COINS

International conference of Life sciences

2018

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CONTENT

ABOUT CONFERENCE

The COINS'18 - 13th international conference of life sciences which gathers not only students and scholars, but also all people that are working in science fields to discuss, learn and share their scientific experience, find new partners, meet key experts and enjoy exciting programme. During the conference participants will get acquainted with scientific innovations, perspectives and most relevant topics in the fields of Molecular biology, Virology, Neurosciences, Biochemistry *etc*.

The COINS also gives an opportunity for BA and MA students and doctorates who are doing their scientific research to present it to a larger audience, get constructive criticism and useful advice.

The COINS'18 is an open scientific environment where everyone interested in life sciences are gathered to build partnerships as well as share and develop new ideas. Conference is based on curiosity, constructive criticism and a wish to improve.

You can find more information about the conference, lecturers, participants and the whole programme in this publication or online: www.thecoins.eu.

SPREAD THE NEWS AND SCIENCE!

FOREWORDS

Dear Colleagues,

The COINS is a unique conference. It is a prime example for the actual implementation of central values of science:

Curiosity: the organizing team and the manifold of students surrounding them strive to communicate and learn from top scientists in the world;

Initiative and courage: it is organized by students in their BSc, while most students at this stage around the world are busy with their personal studies and exams;

Responsibility and organization: the team takes on itself to lead and make this special event happen in a very dedicated and organized manner.

Self criticism and learning from experience: The team is passing the knowledge on how to do it from one generation of students to the next with a well observed improvement;

Enthusiasm together with community and sharing spirit: The excitement as well as community and mutual support atmosphere are evident to every attendee of this great event.

My personal enthusiasm and appreciation of the above bring me back to Vilnius year by year!

Vice Dean Research at the Faculty of Science,
The Hebrew University of Jerusalem
International ambassador of The COINS 2018
Danny Porath

Dear participants of The COINS 2018,

I am honored to be a coordinator of The COINS 2018 conference, because I honestly believe in its purpose. Raising from the initiative of students this conference offers incredible opportunities for all of its participants: for scientists – to meet fellow colleagues or form new partnerships; for students – to listen and be encouraged to follow their path and seek for new knowledge; for companies – to introduce and represent themselves. The COINS gathers the brightest mindsets from all over the world encouraging people to come together and celebrate science.

The COINS allowed me to grow way more than I expected. I hope it helps you grow too.

Sincerely,
Coordinator of The COINS 2018
Justina Mieldažytė

AMBASSADORS

Juozas Rimantas Lazutka
Head of Bioscience institute
Habilitated doctor and professor in Vilnius University
His research mostly concentrates on environmental genotoxicity and population genetics

Danny Porath
Vice Dean for research of the faculty of Science, Etta and Paul Schankerman Professor in molecular biomedicine at the Hebrew University
A founder and coordinator of the Nanoseminar – seminar of the HUJI Center for Nanoscience and Nanotechnology

Egidijus Kinderis
President of Vilnius University Students' Representation
1st Year Master's Student of the Life Sciences Centre

PROGRAMME

FEBRUARY 28, WEDNESDAY

08:00 - 09:00 *Registration and coffee*

09:00 - 09:30 *Opening Ceremony*

Jurgita Petrauskienė, Minister of Education of Lithuania

Juozas Lazutka, Ambassador of The COINS 2018

Egidijus Kinderis, Ambassador of The COINS 2018

Danny Porath, International Ambassador of The COINS 2018

Justina Mieldažytė, Coordinator of The COINS 2018

PANEL DISCUSSION

09:30 - 11:00 **"Human modifications: where is the limit?"**

Moderator: Gintautas Degutis, journalist, technology desk editor at UAB Verslo žinios

Participants:

Prof. Dr. Virginijus Šikšnys – chief scientist and head of Department of protein – DNA interactions

Prof. Dr. (HP) Osvaldas Rukšėnas – head of Department of neurobiology and biophysics

Dr. Jevgenija Vienažindytė – professor of bioethical law at Vilnius university

Gintaras Jonaitis – head of bio-engineering lab at Vilnius Gediminas technical university

11:00 - 11:30	*Coffee break*
	SESSION 1 – MOLECULAR BIOLOGY
11:30 - 12:15	***Keynote speaker presentation*** **Paula Duque** *"A Role for Alternative Splicing in Plant Tolerance to Environmental Stress"* Portugal
12:15 - 12:30	***Student presentation*** **Luca Levay** *"Role of Small GTPases in Autophagy. The Rab3GAP-Rab18 Module"*
12:30 - 12:45	***Student presentation*** **Daina Pamedytytė** *"Overcoming Haemolysis in the Analysis of Circulating miRNA Expression"*
12:45 - 13:00	***Student presentation*** **Kenéz Lili Anna** *"An Unexpected Connection Between Two Related Tethering Complexes"*
13:00 - 13:45	***Keynote speaker presentation*** **Donal O'Carrol** *"RNA Modification and the Immortal Lineage"* United Kingdom
13:45 - 14:45	*Lunch break*

14:45 - 15:30	***Keynote speaker presentation*** **Giedrė Valiulienė** *"Epigenetic Regulation and Leukemia - Research of Novel Biologic, Molecular and Therapeutic Aspects"* Lithuania
15:30 - 15:45	***Student presentation*** **Ilona Užielienė** "Application of Menstrual Blood-derived Mesenchymal Stem Cells for Cartilage Regeneration"
15:45 - 16:00	***Student presentation*** **Réka Somogyi** "Investigating the Role of a Little-known Protein in the Regulation of Lysosomes"
16:00 - 16:15	***Student presentation*** **Martyna Lukoševičiūtė** *"From Pioneer to Repressor: Bimodal foxd3 Activity Dynamically Remodels Neural Crest Regulatory Landscape in vivo"*
16:15 - 17:00	***Keynote speaker presentation*** **Danny Porath** *„Towards Molecular Conductors: Advances in Charge Transport Through Individual DNA and DNA-based Molecules"* Israel
20:00	*Welcome reception*

MARCH 1, THURSDAY

08:00 - 09:00 *Registration*

SESSION 2 – VIROLOGY

09:00 - 09:50 ***Keynote speaker presentation***
Harald zur Hausen
"The Search for Infectious Agents Linked to Human Cancers"
Germany

09:50 - 10:30 ***Keynote speaker presentation***
Ethel-Michele de Villiers zur Hausen
"Characterization of Small Bovine Circular Single-Stranded DNAs which are Infectious for Human Cells"
Germany

10:30 - 11:00 *Coffee break*

SESSION 3 – NEUROSCIENCES

11:00 - 11:45 ***Keynote speaker presentation***
Urtė Neniškytė
"Synapse Elimination: the Mechanisms of Glial-Cell-Dependent Synaptic Pruning"
Lithuania

11:40 - 12:15 **Dr. Birutė Railienė** – Wroblewski Library of the Lithuanian Academy of Sciences
"Standing on the shoulders of giants: A founder of biochemistry - Jedrzej Sniadecki (1768–1838), Professor of Vilnius University"

12:15 - 12:30	***Student presentation*** **Artūras Bružas** *"The Role of Phosphatidylserine in Synapse-microglia Interaction"*
12:30 - 12:45	***Student presentation*** **Kristina Jevdokimenko** *"MFG-E8 and Active Caspase-3 in Synaptic Pruning by Microglia"*
12:45 - 14:30	*Coffee break*
12:45 - 14:30	***Company Fair***
13:30 - 14:30	*Lunch break*
14:30 - 15:15	***Keynote speaker presentation*** **Ludmilla Morozova – Roche** *"Role of Pro-Inflammatory S100 Proteins in Amyloid-Inflammatory Cascade in Neurodegenerative Amyloid Diseases"* Sweden
15:15 - 15:30	***Student presentation*** **Péter Benkő** *"The Role if Rab39 in Lysosome Biogenesis"*
15:30 - 15:45	***Student presentation*** **Miglė Kalvaitytė** *"The Impact of Scaffold Microstructure on Dental Pulp Stem Cells Osteogenic Differentiation"*

15:45 - 16:00 ***Student presentation***
Győző Szenci
"Ykt6 Acts as a Non-canonical SNARE in Autophagosome-lysosome Fusion"

16:00 - 16:45 ***Keynote speaker presentation***
Svenja Caspers
"Decoding the Organization of the Human Brain Across the Scales"
Sweden

MARCH 2, FRIDAY

08:30 - 09:30 *Registration*

*SESSION 4 – **BIOCHEMISTRY***

09:30 - 10:15 ***Keynote speaker presentation***
Juozas Nainys
„Full-Stack Single-Cell Transcriptomics Provides a Deeper Understanding of Cancer Metastasis"
Lithuania

10:15 - 11:15 ***Keynote speaker presentation***
Erwin Neher
"Ion Channels: Their Discovery, their Function and their Role in Medicine and Pharmacology"
Germany

11:15 - 11:30	***Student presentation*** **Gražvydas Lukinavičius** "Biocompatible Probes for Imaging of Cellular Structures"
11:30 - 11:45	***Student presentation*** **Giulio Preta** *"Studying Lipid Rafts: Six Degrees of Inner Turbulence"*
11:45 - 13:30	*Coffee break*
11:45 - 13:30	***Poster Session***
12:30 - 13:30	*Lunch break*
13:30 - 14:15	**Vilnius-Lithuania, Grand prize winner of the iGEM competition** *"SynOri: a framework for multi-plasmid systems"*
14:15 - 14:30	***Student presentation*** **Diana Ikasalaitė** "In vitro Repair of 5-alkylcytosines"
14:30 - 14:45	***Student presentation*** **Gediminas Skvarnavičius** "Investigating Ligand Binding Induced Changes In Protein Volume Using High Pressure Spectrofluorimetry"

14:45 - 15:30	***Keynote speaker presentation*** **Ramunas Martin Vabulas** *"How Mammalian Cells Sense Proteostasis Stress"* Germany
15:30 - 16:00	*Awards and Certificates*
20:00	*Closing Event*

KEYNOTE SPEAKERS

Paula Duque (Portugal)
Group Leader at the Instituto Gulbenkian de Ciência (IGC), Oeiras

"A Role for Alternative Splicing in Plant Tolerance to Environmental Stress"

duquep@igc.gulbenkian.pt

Alternative mRNA splicing, which generates multiple transcripts from the same gene, is a highly prevalent posttranscriptional gene expression regulation mechanism in eukaryotic genomes. While alternative splicing has been shown to determine key biological processes in animal systems and its misregulation is associated with many human diseases, the functional significance of this molecular mechanism in plants remains poorly understood. As a potent generator of proteome diversity that is markedly affected by external cues in plants, alternative splicing is likely to play an important role in the ability of these sessile organisms to cope with environmental stress.

Arginine/serine-rich (SR) proteins are highly conserved RNA-binding factors that play crucial roles in the regulation of alternative splicing. I will describe how our lab's characterization of these splicing factors in the model plant *Arabidopsis thaliana* is providing functional links between alternative splicing and plant stress responses, particularly those mediated by the abscisic acid (ABA) hormone, which is crucial in conferring tolerance to the most pervasive causes of loss of crop productivity worldwide.

Dónal O'Carroll (United Kingdom)

MRC Centre for Regenerative Medicine, Institute of Stem Cell Research, School of Biological Sciences, University of Edinburgh, Edinburgh

"RNA Modification and the Immortal Lineage"

Several stages of both male and female gametogenesis are transcriptionally quiescent; thus the germline is heavily reliant on post-transcriptional regulation of gene expression. This is especially relevant for the female germline, where the post-transcriptional utilization of the maternal transcriptome underpins meiotic maturation, fertilization and early embryonic development. The variety and abundance of RNA modifications coupled with the realization of their regulatory importance has given rise to the nascent field of epitranscriptomics. Here we sought to understand if epitranscriptomic regulation contributes to the assembly or metabolism of the mammalian maternal transcriptome. The presentation will focus on the importance of the N^6-methyladenosine-reader YTHDF2 as well as the TUT4/7 3' uridylases in oogenesis and early zygotic development.

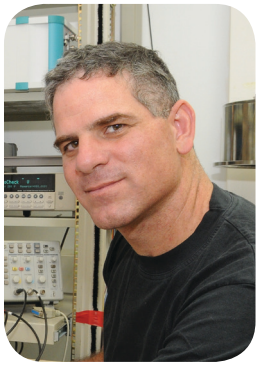
Giedrė Valiulienė (Lithuania)
PhD student at the Department of Molecular Cell Biology, Life Sciences Center, Vilnius University, Vilnius

"Epigenetic Regulation and Leukemia – Research of Novel Biological, Molecular and Therapeutic Aspects"

ASeveral thousands of people worldwide are being diagnosed with acute promyelocytic leukemia (APL) annually. Usually APL patients are being treated with anthracyclines and RA (all trans retinoic acid) combination. It has been showed that RA is able to induce APL cell differentiation into mature granulocytes. However, despite the general success of RA therapy, RA treatment resistant cases still remain a serious issue. It has been demonstrated that epigenetic remodelling could overcome APL resistance to RA. Moreover, it is known that limited efficacy of RA treatment in other types of leukemias does also rely largely on epigenetic factors.

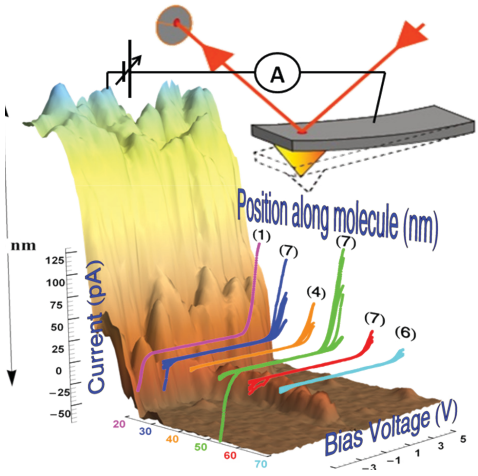
In this study we aimed to evaluate the impact of epigenetic regulation of human APL cells induced to granulocytic differentiation. We have shown that histone deacetylase inhibitor (HDACi) belinostat (PXD101), and histone methyltransferase inhibitors (HMTi) BIX-01294 and 3-deazaneplanocine A facilitate RA-induced human APL cell NB4 and HL-60 differentiation into mature granulocytes. Results obtained support the efficacy of APL epigenetic therapy in vitro. In addition, we developed the murine APL xenograft model and showed that used agents prolong APL xenograftic mice life span and protect them against tumour formation. Furthermore, we have evaluated the effect of epigenetic therapy on histone modification changes in APL xenograftic mice tumours and tissues. Results obtained in our study could be valuable for future APL epigenetic therapy clinical studies as well.

Danny Porath (Israel)
Professor of Chemistry, Etta and Paul Schankerman Chair of Molecular Biomedicine at the Institute of Chemistry, Jerusalem;
Vice Dean Research at the Faculty of Science, The Hebrew University of Jerusalem, Jerusalem



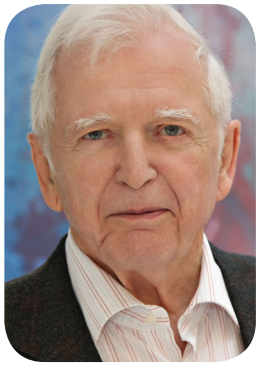
"Towards Molecular Conductors: Advances in Charge Transport Through Individual DNA and DNA-based Molecules"

danny.porath@mail.huji.ac.il



DNA is primarily and with no doubt the most important biological molecule. Its double-strand recognition, as well as the ability to control its sequence and manipulate its structure open a multitude of ways to make it useful for molecular electronics. Step by step we improve the synthesized constructs and the measurement methods of single DNA-based molecules. In this lecture I will review the field and report on our progress in producing and measuring DNA-based building blocks towards the construction of DNA-based programmable circuits.

Harald zur Hausen (Germany)



Professor Emeritus at the German Cancer Research Centre, Heidelberg

"The Search for Infectious Agents Linked to Human Cancers"

Nobel Prize in Physiology or Medicine 2008

During the past 5 decades a number of infectious agents have been linked to specific human cancers. These include viruses (e.g. *Epstein-Barr Virus*, *Human Herpesvirus type 8*, *Hepatitis B Virus*, *Hepatitis C Virus*, *specific human papillomavirus types*, *T-lymphotropic retrovirus*, *human immunodeficiency virus*, *Merkel-cell polyomavirus*) as well as bacteria (*Helicobacter pylori*) and parasitic infections (*Schistosoma hematobium*, *Opisthorchis viverinni*). All together, they account for approximately 21 % of the global cancer incidence with wide geographic variations. The identification of these agents had major impact for the development of novel strategies for cancer prevention.

We analyzed the question whether it is worthwhile to study additional human cancers linked to these infections. Initially this was based on epidemiological observations by correlating incidence pattern with specific nutritional habits. This resulted in the suggestion the consumption of beef and dairy products of Eurasian cattle significantly contributes to the risk for colon, breast and prostate cancers. Subsequently we isolated and sequenced a large number of small circular single-stranded DNA from sera and dairy products of dairy cows, named bovine meat and milk factors (*BMMF*) Those which have been tested by now are genetically active in human cells and replicate in specific human cell lines. A high percentage of healthy humans develop antibodies against the major protein (rep) of one subgroup. Four of these molecules have been isolated from materials of patients with multiple sclerosis.
Presently, experiments are being conducted to study their role in malignant tumors and in neurodegenerative diseases.

Ethel-Michele de Villiers zur Hausen (Germany)

Researcher at the German Cancer Research Centre, Heidelberg

"Characterization of Small Bovine Circular Single-stranded DNAs which are Infectious for Human Cells"

Over the years a large variety of laboratory methods have been used to isolate unknown infectious agents. The conservative methods included partial purification, susceptibility for replication in cell culture, electron microscopy and/or density gradient centrifugation, just to mention a few. These were all in part biased and limited to available experimental methods. With the advent of the polymerase chain reaction and sequencing, many viruses could initially be identified via their genomic characteristics, followed, if possible by biological analyses. The most recent application of whole genome sequencing of samples with or without partial purification, gives insight into a whole new spectrum of infectious agents. This novel information also stresses the point that historical divisions between animal, plant and bacterial "viruses" are outdated. Several examples will be reviewed in short.

In our present study a combination of density purification and genomic amplification led to the identification of circular single-stranded DNA molecules in bovine serum and dairy products (BMMF-bovine milk and meat factors), as well as serum and brain samples from patients with multiple sclerosis. These isolates were grouped into 4 groups according to their relatedness. Several characteristics were identified which had previously been described for bacterial plasmids or plant viruses or human viruses. These will be discussed in more detail. Replication in human cells of the DNA isolates with characteristics of bacterial plasmids was demonstrated by transfection.

Urtė Neniškytė (Lithuania)

Marie Sklodowska Curie Experienced Researcher at the Department of Neurobiology and Biophysics, Life Science Center, Vilnius University, Vilnius

"Synapse Elimination: the Mechanisms of Glial-Cell-Dependent Synaptic Pruning"

The final stage of brain development is associated with the generation and maturation of neuronal synapses. However, the same period is also associated with a peak in synapse elimination — a process known as synaptic pruning — that has been proposed to be crucial for the maturation of remaining synaptic connections. Recent studies have pointed to a key role for glial cells in synaptic pruning in various parts of the nervous system and have identified a set of critical signalling pathways between glia and neurons. At the same time, brain imaging and post-mortem anatomical studies suggest that insufficient or excessive synaptic pruning may underlie several neurodevelopmental disorders, including autism, schizophrenia and epilepsy. I will present current data on the mechanisms of glial-cell-dependent synaptic pruning and outline their potential contribution to neurodevelopmental disorders.

Ludmilla Morozova – Roche (Sweden)

Professor in Medical Biophysics at the Department of Medical Biochemistry and Biophysics, Umeå Univeristy, Umeå

"Role of Pro-Inflammatory S100 Proteins in Amyloid-Inflammatory Cascade in Neurodegenerative Amyloid Diseases"

Pro-inflammatory S100A8/A9 proteins are increasingly recognized as important contributors to inflammation-related neurodegeneration. These are small Ca^{2+}-binding EF-hand proteins involved in diverse cellular processes such as cell survival, proliferation, differentiation and danger signaling. The expression level of S100 proteins and among them S100A8 and S100A9 is significantly increased in many types of cancer, inflammatory, neurodegenerative and autoimmune diseases and they are implicated in the numerous disease pathologies. We have demonstrated that S100 proteins are intrinsically amyloidogenic and S100A6, S100A8 and S100A9 are able to form amyloids both in vitro and in vivo in cell models and in neurodegenerative diseases. The specific mechanisms of S100A9 action in Alzheimer's disease and traumatic brain injuries are discussed, where it contributes to amyloid plaques formation and neural cytotoxicity together with Aβ, potentially playing a leading role in these processes. Recently we have demonstrated that S100A9 can serve as a robust biomarker differentiating early stages of cognitive impairment in Alzheimer's disease, especially in conjunction with others such as Aβ(1–42) and tau-proteins, which further signifies its involvement in Alzheimer's disease pathology. The area of interests include also the *in vitro* studies of the mechanisms and pathways of S100 self-assembly, including kinetic and structural studies, the features of amyloid precursor state and regulatory factors of the amyloid self-assembly process.

Svenja Caspers (Sweden)

Associate Professor for Human Brain Connectivity at the C. and O. Vogt Institute for Brain Research, Heinrich-Heine University, Düsseldorf

"Decoding the Organization of the Human Brain across the Scales"

The human brain enables such diverse abilities such as language, action control, decision making, attention, memory or emotions and personality, some of which are thought to be uniquely human. Understanding the organization of the human brain on multiple levels, from single neurons and their axons to whole brain networks and large fiber bundles is one of the current challenges and fascinating endeavors of the 21st century. Integrative and encompassing knowledge of the healthy human brain and its normal variability is a crucial prerequisite for finally disentangling pathological alterations due to neurodegenerative and psychiatric diseases, major burdens in modern society.

Modern systems neuroscience provides novel insights into human brain organization across the scales, local cellular and fiber architecture to large-scale cognitive networks. While microscopic techniques on postmortem brain tissue enable high-resolution views on local architecture of neurons, neurotransmitter receptors and fibers, state-of-the-art magnetic resonance imaging (MRI) techniques allow for investigation of fiber bundles, cognitive networks during task performance as well as in the resting state and gross brain structure. Integrating these different scales is of utmost importance for an integrated understanding of the human brain and one of the aims of the EU-FET Flagship "Human Brain Project" (HBP).

Juozas Nainys (Lithuania)
PhD student at the Sector of Microtechnologies, Life Science Center, Vilnius University, Vilnius

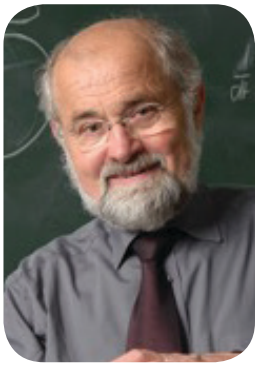
"Full-Stack Single-Cell Transcriptomics Provides a Deeper Understanding of Cancer Metastasis"

Single cell transcriptomics is a novel and rapidly developing field. It is fast becoming clear that single cell technologies are powerful methods for detailed gene expression analyses, new cell type identification as well as mapping complex cell populations. Recently described high-throughput microfluidic protocols for single cell transcriptomics have substantially reduced labour and reagent costs which makes it possible to analyse tens of thousands of single cells.

Here we have applied droplet microfluidic based scRNAseq technology to gain deeper understanding of cancer metastasis. Epithelial to mesenchymal transition (*EMT*) is an important process in cancer metastasis. During EMT cells gradually transition through various intermediate states. It is thought that cancer stem cell may be formed during EMT. To thoroughly analyse this process, we have profiled over 20,000 single cells during 12 days of EMT. To gain more insight into this multidimensional dataset we have developed a novel algorithm called MAGIC (*Markov Affinity-based Graph Imputation of Cells*). This algorithm uses data diffusion to impute missing values and recover gene networks within single cells. To validate our algorithm, we have performed an independent biological perturbation. We have additionally profiled over 10,000 single cells under *Zeb1* transcription factor overexpression. Results provide validation for the MAGIC algorithm as well as the formed hypothesis.

Work described here reveals the power and utility of high throughput single-cell analyses. New generation microfluidic platform together with the novel algorithms allow for unbiased analyses of intricate gene networks within single-cells.

Erwin Neher (Germany)



Emeritus Director at the Department of Membrane Biophysics, Max Planck Institute for Biophysical Chemistry, Göttingen

"Ion Channels: Their Discovery, their Function and their Role in Medicine and Pharmacology"

Nobel Prize in Physiology or Medicine 1991

The concept of bioelectricity emerged in the late 18th century, based on the experiments of Galvani and Volta. 60 years ago Hodgkin & Huxleyshowed that the nerve impulseis a result of permeability changes of the nerve membrane. This provoked the question what the molecular mechanisms of such permeability changes might be. In 1976 Bert Sakmann and myself were able to show that so-called ion channels – proteins, which gate ion fluxes across membranes- mediate these responses.

Research over the last 40 years has shown, that ion channels are not only present in electrically excitable cells, such as in nerve and muscle, but also in basically all celltypes of our body, mediating a variety of physiological functions. We now know, that they are prime drug targets,and that dysfunction of ion channels underliesa variety of diseases.

Ramunas (Martin) Vabulas (Germany)

Group Leader at the Buchmann Institute for Molecular Life Sciences, Goethe University, Frankfurt

"How Mammalian Cells Sense Proteostasis Stress"

Cells respond to protein misfolding and aggregation in the cytosol by adjusting gene transcription and a number of post-transcriptional processes. In parallel to functional reactions, cellular structure changes as well; however, the mechanisms underlying the early adaptation of cellular compartments to cytosolic protein misfolding are less clear. We showed that the mammalian ubiquitin ligase C-terminal Hsp70-interacting protein (CHIP), if freed from chaperones during acute stress, can dock on cellular membranes thus performing a proteostasis sensor function. We reconstituted this process *in vitro* and found that mainly phosphatidic acid and phosphatidylinositol-4-phosphate enhance association of chaperone-free CHIP with liposomes. HSP70 and membranes competed for mutually exclusive binding to the tetratricopeptide repeat domain of CHIP. At new cellular locations, access to compartment-specific substrates would enable CHIP to participate in the reorganization of the respective organelles, as exemplified by the fragmentation of the Golgi apparatus (effector function).

ORAL PRESENTATIONS

Luca Lévay

"Role of Small GTPases In Autophagy. The Rab3GAP-Rab18 module"

LUCA LÉVAY, Szabolcs Takáts, Luca Lévay, Péter Lőrincz, Gábor Juhász

Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Budapest, Hungary

Autophagy which means 'self-eating', is an evolutionarily conserved degradation pathway in eukaryotes. During this process, superfluous or aberrant organelles are sequestered into a double-membrane vesicle, called autophagosome, that eventually fuses with the lysosome, so in that way the enclosed contents can be degraded and the resulting macromolecules can be recycled. This process is essential for cellular homeostasis and it's disfunction is linked to several diseases and to aging as well.

However, autophagosome-lysosome fusion is a key step of autophagy, our knowledge about its genetic regulation is still limited. During the autophagic process the cellular endomembrane system undergoes extensive transformation. Membrane dynamics is mainly regulated by small GTPases of the Rab protein family. Rabs are switch-like proteins which have a GTP bound active and a GDP bound inactive conformation. This nucleotide binding status is dependent of the presence of activating and inactivating cofactrors,GEFs (guanine nucleotide exchange factor), and GAPs (GTPase-activating protein) respectively.

Our research focuses on the Rab3GAP2 protein that forms a heterodimeric complex with Rap3GAP1. Rab3GAP1/2 complex is multi-modal Rab cofactor as it can function as a GAP for Rab3, but

it can also act as a GEF for Rab18. During our study in Drosophila melanogaster, we found that the loss of Rab3GAP2 inhibits autophagic degradation. In addition, with light and electron microscopy we showed that in Rab3GAP2 loss of function mutant cells, the lysosomal system is disorganized, and autophagosomes accumulate due to impaired fusion events. We also found that silencing the Rab18 gene inhibits autophagy in a similar way to Rab3GAP2. These results suggest, that the Rab3GAP-Rab18 module represents a novel regulatory step in autophagosome-lysosome fusion. Our aim is to discover the exact mechanism by which these proteins regulate this essential step of autophagy.

Daina Pamedytytė

"Overcoming Haemolysis in the Analysis of Circulating miRNA Expression"

DAINA PAMEDYTYTĖ[1], Vaida Simanavičienė[1], Aurelija Žvirblienė[1], Birutė Žilaitienė[2]

1. Department of Immunology and Cell Biology, Life Sciences Center, Vilnius University, Vilnius, Lithuania

2. Lithuanian University of Health Sciences, Kaunas, Lithuania

The development of molecular markers of papillary thyroid carcinoma (PTC) recurrence such as miRNAs has the potential to improve the clinical management of patients by assisting in risk stratification. Therefore, our aim was to identify specific circulating miRNAs that could be used as biomarkers of PTC recurrence. However, the quantification of miRNAs from plasma is difficult due to haemolysis which may have a substantial impact on detected miRNA level. Therefore, in this study we used different approaches to overcome haemolysis-associated inaccuracies in order to measure true circulating miRNA levels.

We selected 3 miRNAs (miRNA- 146b, -222 and -21) which are overexpressed in PTC compared to healthy thyroid tissue and investigated the effect of haemolysis on the levels of these miRNAs by artificially introducing haemolysis in non-haemolysed plasma. We found out that miRNA-21 level depended greatly on haemolysis, while miRNA-146b and -222 were less affected. Then we measured miRNA levels in 65 blood plasma samples and found that using an absorbance at 414 nm of 0,25 as a cut-off to distinguish between the haemolysed and non-haemolysed plasma significantly decreased the variability in all three miRNAs levels ($p < 0,05$). Nine detected haemolysed samples were eliminated from further analysis and the rest 56 plasma samples were divided in 4 groups (samples collected before tumour removal, after removal, samples from patients with benign thyroid nodules and from healthy people). However, the levels of all analysed miRNAs were very similar between the groups. Hence, more research is needed to draw a conclusion about the potential of circulating miRNA-146b, -222 and -21 as PTC biomarkers.

This study was a part of project SEN-14/2015 supported by the Lithuanian Research Council.

Kenéz Lili Anna

"An Unexpected Connection between two Related Tethering Complexes"

LILI KENÉZ, Péter Lőrincz, Sarolta Tóth, Zsófia Simon-Vecsei, Gábor Juhász

Introduction: During endocytosis several homotypic and heterotypic fusions can be observed in the cells. Membrane specific Rab GTPases (e.g., Rab5 and Rab7), tethering factors and SNARE proteins are needed to anchor and fuse vesicles together. The yeast CORVET and HOPS multi-subunit tethering complexes are formed of a shared core (containing 4 proteins) and two complex specific Rab-binding subunits (CORVET: Vps8 and Vps3, HOPS:Vps41 and Vps39) at opposing ends.

Aim: Our laboratory has previously shown that HOPS is similar to yeast in Drosophila and is needed for lysosmes to fuse with late endosomes or autophagosomes. Interestingly a smaller, 4 subunit containing miniCORVET complex was found, which promotes homotypic fusions of early endosomes and contains only one specific subunit (Vps8). Although CORVET is specific for early endosomal fusions, data from yeast suggested that overexpression of Vps8 may have an effect on HOPS related functions, which we wanted to examine a metazoan model.

Materials and methods: We used UAS-Gal4 system for the overexpression of Vps8 in a subset of fruit fly cells (such as larval nephrocytes and fat cells). Dissected larval tissues were immunostained against autophagic or endocytic markers to evaluate autophagy or endocytosis. Obtained results were confirmed by electronmicroscopy. Western blot assays were used to estimate autophagic flux.

Result and Conclusion: As expected Vps8 overexpression caused a similar phenotype to HOPS mutant cells. These cells accumulated Atg8 and p62, and autophagosomes, indicating autophagic flux impairment. Similarly, late endosomal clearance is also blocked. In contrast to Vps8, overexpression of its HOPS specific counterpart (Vps41) had no specific effects. Taken together our data, we suggest that Vps8 competes with Vps41 for the same binding partners to form miniCORVET versus HOPS.

Ilona Užielienė

"Application of Menstrual Blood-derived Mesenchymal Stem Cells for Cartilage Regeneration"

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TThe degradation of cartilage tissue caused by trauma or chronic and progressive degenerative joint disease (e.g., osteoarthritis) has become a global problem for which no efficient therapy is available nowadays. Mesenchymal stem cells (MSCs) with their multipotent differentiation capability seem promising candidates for cartilage regeneration. Although the first time MSCs were found in bone marrow, nowadays they can be isolated from almost all human tissues, including menstrual blood (MenSCs). MenSCs were capable to differentiate into several directions much like classical bone marrow MSCs (BMMSCs). Although MenSCs obtaining methods have many advantages comparing to other tissues MSC (they are inexpensive, easily-accessible), these cells are very little investigated so far. The aim of this study was to compare stem cell properties of MenSCs and BMMSCs focusing on

the investigation of chondrogenic differentiation potential, using different growth factors (Activin A and TGF-β3), and the impact of intracellular calcium signaling in it, as intracellular Ca^{2+} is known to improve cell differentiation potential.

We isolated MenSCs and BMMSCs from healthy donors and estimated their stem cell surface markers by flow cytometry. Proliferation capacity of both cell types has been investigated using proliferation kit CCK-8. Furthermore, we estimated intracellular calcium by fluorescent dye Cal-520 and fluorescence microscope. Finally, the adipogenic (Oil-Red staining) and osteogenic (Alizarin Red staining) differentiations of MenSCs and BMSCs were investigated by light microscope, whereas chondrogenic differentiation (activin A and TGF-β3stimulation) was investigated by immuno- and histochemical staining with collagen II antibodies and safranin O, respectively.

Our results demonstrate that in contrast to BMMSCs, MenSCs exhibited different expression of surface markers, and higher proliferative capacity. MenSCs also showed higher level of intracellular calcium compared to BMMSCs. Adipogenic differentiation of MenSCs was weaker, whereas osteogenic differentiation capacity was higher compared to BMMSCs. Chondrogenic differentiation with TGF-β3 was similar for both cell types, while Activin A-induced chondrogenic differentiation was remarkably higher in MenSCs than in BMMSCs. We conclude that MenSCs exhibit similar stem cell properties to BMMSCs and may be considered as a potential stem cell source for further studies of chondrogenic differentiation *in vitro*.

Réka Somogyi

"Investigating the Role of a Little-known Protein in the Regulation of Lysosomes"

The proper lysosomal maturation and function are essential in the life of cells. Small GTPase proteins, for example Rabs, are important regulators of membrane trafficking, they have important function in lysosomal degradation processes when lysosomes fuse with endosomes or/and autophagosomes which contain materials have to be degraded. It is shown that a littleknown protein, C11.1 can interact with Rab7, an important regulator of lysosomal fusion events (Gillingham, 2014). In our work we focused on autophagy, the self-degradative process of the cells in which the cytoplasmic material containing autophagosomes fuse with lysosomes to create autolysosomes and degrade the sequestrated material.

We examined whether C11.1 plays role in autophagy and we found that autophagic and lysosomal compartments are disrupted in the absence of this protein. Further we observed aberrant lysosomes with ultrastructural analysis in the fat body of starved Drosophila larvae. Based on these results we concluded that C11.1 participate in the regulation of lysosome maturation and lysosomal fusion events.

Martyna Lukoševičiūtė

"From Pioneer to Repressor: Bimodal foxd3 Activity Dynamically Remodels Neural Crest Regulatory Landscape *in vivo*"

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Introduction: The neural crest (NC) is a multipotent embryonic stem cell-like population that gives rise to a wide range of derivatives, including sensory neurons and glia, cartilage, bone and connective elements of craniofacial skeleton and the vast majority of body's pigmentation. Multi-modular gene regulatory network (GRN) controls different phases of NC ontogeny including specification, epithelial-tomesenchymal transition, migration and terminal differentiation into numerous derivatives.

Aim: Early in development, the forkhead/winged helix transcription factor foxd3 is expressed in a broad domain that includes NC precursors, the *bona fide* pre-migratory NC progenitors and later, a subset of NC derivatives. Foxd3 has been shown to mediate late NC lineage decisions, but its putative role during NC specification remains elusive. Here we aim to tackle foxd3 regulatory activity throughout NC specification, differentiation and early migration.

Methods: To elucidate the mechanistic mode of action of foxd3, we analysed its genome-wide binding profiles across multiple stages of embryonic development using our novel biotagging approach allowing to biotinylate foxd3 protein *in vivo* (biotin-ChIP). We integrated this data within the context of NCspecific transcriptional and epigenomic profiles (ATAC-seq, H3K27Ac ChIP-seq, RNA-seq) of foxd3- mutant versus control cells at each corresponding developmental stage in order to define the gene regulatory circuits controlling NC development progression.

Results: Our analyses revealed that during early steps of NC formation, foxd3 acts globally as a pioneer factor to prime the onset of genes regulating NC specification and migration by re-arranging the chromatin landscape, opening associated NC enhancers and reshuffling nucleosomes. Strikingly, foxd3 then gradually switches from an activator to its canonical role as a transcriptional repressor. Taken together, these results demonstrate that foxd3 acts bimodally in the NC as a switch from ‚permissive' to ‚repressive' nucleosome/chromatin organisation to maintain ‚stemness' and define cell fates.

Conclusion: Our results provide global regulatory information allowing to build the representation of the minimal GRN circuits controlling NC specification, bringing us one step closer to 'knowing how' to instruct stem cells to acquire NC cell fate.

Artūras Bružas

"The Role of Phosphatidylserine in Synapse-microglia Interaction"

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One of the key features of the postnatal nervous system development is neuronal structure refinement and excess synapse removal via synaptic pruning. Synaptic pruning is crucial for healthy brain and proper cognitive development as pruning impairment might lead to various neurological disorders. One of the most promising signaling molecules in regulation of this process, is phospholipid phosphatidylserine (PtdSer), a well-known "eat-me" signal that is exposed on extracellular layer of cell membrane and thus attracts microglia, the resident brain macrophages that removes target structures by phagocytosis. Milk fat globule-EGF factor 8 protein (MFG-E8) is a PtdSer-specific opsonin that coats PtdSer-exposing structures and mediates the engulfment of targets.

Importantly, recently it has been shown that microglia also plays a major role in synaptic pruning. The aim of this study was to investigate microglia interactions with pre- and postsynaptic structures in CA1 region of developing hippocampus and the role of PtdSer in guiding microglia to target synapses.

In this study we used fixed brain slices from *Thy1*::GFP;*Cx3cr1*::tdTomato mice and stained them immunohistochemically for MFG-E8. By using laser scanning

confocal microscopy we looked for the colocolization of MFG-E8, microglia and neuronal signals and evaluated the effect of the presence of MFG-E8 for the interactions of dendritic spines and axonal boutons with microglia.

Synaptic structures labelled with MFG-E8 were contacted by microglia 2-3 times more often than those without MFG-E8. Presynaptic boutons containing MFG-E8 were contacted significantly more often than MFGE8-containing postsynaptic spines.

Kristina Jevdokimenko

"MFG-E8 and Active Caspase-3 in Synaptic Pruning by Microglia"

KRISTINA JEVDOKIMENKO, Augustė Vadišiūtė, Cornelius Gross, Urtė Neniškytė
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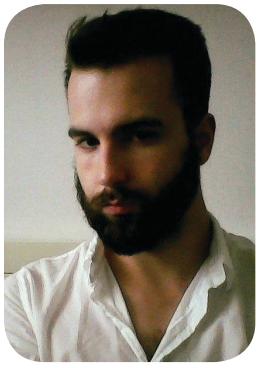
Microglia are brain resident macrophages that contribute to synaptic pruning during brain development. The mechanism of synapse elimination might be mediated by microglial recognition of phospholipid phosphatidylserine (PtdSer) on neuronal synapses. This process needs to be strictly regulated, since both underpruning and overpruning might cause several neurodevelopmental diseases, such as autism spectrum disorders or schizophrenia. We have previously found that microglia-synapse interaction involves PtdSer exposure and binding of PtdSer-specific opsonin milk fat globule-EGF factor 8 (MFG-E8). Synaptic pruning required functional phospholipid scramblase XK-related protein 8 (Xkr8), which promotes PtdSer transition to the extracellular layer of plasma membrane after cleavage by active caspase-3 (act-casp-3).

Therefore we compared PtdSer exposure and active caspase-3 expression in wild-type and Xkr8-deficient hippocampus of developing mouse (at postnatal day 15).

We used MFG-E8 as a surrogate marker of PtdSer exposure and subjected confocal images of immunohistochemically labelled brain slices to 3D image analysis. This analysis was performed in three levels – first, to measure MFG-E8 and act-casp-3 signal in the field of view of CA1 part of hippocampus, second, to measure individual signals of MFG-E8, act-casp-3 in the dendrites of hippocampal CA1 pyramidal neurons, third, to evaluate the colocalization between MFG-E8, act-casp-3 and dendrite.
Our findings show, that Xkr8 may promote act-casp-3-dependent PtdSer exposure on the surface of neurons thus presenting an "eat-me" signal for microglia-mediated synaptic pruning.

Péter Benkő

"The Role of Rab39 in Lysosome Biogenesis"



PÉTER BENKŐ, Péter Lőrincz, Lili Kenéz, Gábor Juhász

Introduction: Membrane fusion is conducted by a complex supramolecular machinery within eukaryotic cells. Small GTPases of the Rab family are part of this machinery and provide membranes with identity by binding specific tethering factors and motor proteins. This function ensures proper targeting and fusion of membranes within the intracellular membrane trafficking network. The subject of our study, Rab39 is a Golgi-associated Rab protein suspected to be involved in the endolysosomal-autophagosomal pathway, as it has been shown to bind Unc- 104 which is a *Drosophila* ortholog of the mammalian

kinesin-3 KIF1A, as well as a PLEKHM family member that recruits kinesin-1 to lysosomes, and also Ema, a factor involved in endosomal fusions which binds HOPS, one of the Class C core endosomal tethering complexes.

Aim: The goal of our study is to characterize Rab39 function in *Drosophila* and provide an insight into its role in lysosomal biogenesis. Human Rab39B has been linked to Waisman syndrome, a neurodegenerative disease related to Parkinson's, therefore biomedical implications may be present.

Materials and methods: Fluorescent imaging was used to determine most phenotypes, images show results of immunocytochemistry, vital stainings and fluorescent reporter expressions. *Drosophila melanogaster* larval fat bodies and garland nephrocytes were used as model systems. Additional biochemical methods such as Western blot were employed.

Results: We have determined that Unc-104 indeed functions as a kinesin in *Drosophila*, as its depletion leads to perinuclear accumulation of lysosomes. Rab39 depletion seems to cause a decrease in lysosome size in larval fat cells, and leads to perinuclear accumulation of lysosomes in garland nephrocytes, coupled with an enlarged late endosomal compartment at the cell periphery.

Conclusion: These results suggest an important role for Rab39 in lysosomal fusions and positioning, functions that are set to be in our focus in upcoming experiments.

Miglė Kalvaitytė

"The Impact of Scaffold Microstructure on Dental Pulp Stem Cells Osteogenic Differentiation"

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Introduction: Tissue engineering is a multidisciplinary science orientated to the development of artificial tissue or organ that could be used for treatment. Artificially engineered bone tissue grafts are needed in medicine for bone regeneration. Artificial bone creation requires appropriate cell source and scaffold selection, as well as determination of optimal cell culture conditions, which have not been elucidated yet.

Aim: Evaluate the impact of polylactic acid (PLA) scaffolds' microstructure on rat's dental pulp stem cells (DPSC) osteogenic differentiation *in vitro*.

Materials and methods: DPSC were isolated from rat's dental pulp by outgrowth from intact tissue and purified with magnetic beads coated with antibodies against cell surface marker CD44. These cells were characterized by flow cytometry and their multipotency was examined by differentiation assay. Two differently microstructured PLA scaffolds – wavy and porous, were created by 3D printing. DPSC proliferation on PLA scaffolds was evaluated by measuring DNA concentration in cells' lysate with DAPI dye. DPSC osteogenic differentiation on analyzed scaffolds was evaluated by staining mineralized matrix with Alizarin Red S and measuring alkaline phosphatase activity. The expression of Runx2, osteopontin (OPN) and osteocalcin (OCN) genes were

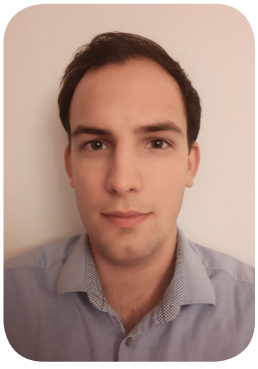
evaluated at different time points using reverse transcription and qPCR.

Results: Osteogenic differentiation results indicated that wavy PLA scaffolds were more suitable for matrix mineralization; however, alkaline phosphatase activity increased the most in cells, seeded on porous scaffolds. Gene expression analysis showed, that Runx2 and OPN genes' transcription is earlier activated in cells, grown on wavy scaffold; at further time points no difference between two analyzed scaffolds has been found. OCN gene expression changes wasn't detected. It was also showed, that cell's environment and scaffold's microstructure is sufficient enough to induce DPSC osteogenic differentiation.

Conclusion: PLA scaffolds are suitable for rat's DPSC proliferation and osteogenic differentiation. Moreover, surface topography influence DPSC osteogenic differentiation capacity.

Győző Szenci

"Ykt6 Acts as a Non-canonical SNARE in Autophagosome-lysosome Fusion"



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Autophagy is a highly conserved eukaryotic catabolic pathway, which is responsible for the degradation of the damaged or superfluous cellular materials. During the process the phagophore encloses the cargo, seales to double membrane autophagosomes, which ultimately fuses with lysosomes. In the formed acidic autolysosomes, the uptaken material gets degraded and then the released macromolecular building blocks are reused by the cell. For the eukaryotic membrane fusion events the consecutive action of Rab GTPases, tethering factors and the SNARE proteins are needed. During the fusion reactions, the SNAREs in opposing membranes form a trans-SNARE complex, which provides the mechanical force for the final step of the fusion reaction. The trans-SNARE complex responsible for autophagosome-lysosome fusion is composed of Syntaxin 17, Snap29 and VAMP7. Surprisingly, we found that the presence of Ykt6, an other SNARE protein, is also nescessary for the proper autophagosome clearance in Drosophila. We revealed that VAMP7 and Ykt6 compete with each other for the interaction with Syx17 and Snap29. In this competition Ykt6 appeared the less competitve component, and we demonstrated that the SNARE motif of Ykt6 binds much weaker to Syntaxin17 and Snap29, than VAMP7 do. We also revealed that both Ykt6 and VAMP7 N-terminal longin domains are able to interact with HOPS tethering complex. These data suggest that Ykt6 acts as a non-canonical, regulatory SNARE in the autophagosome-lysosome fusion through the recruitment of the other fusion factors to the fusion sites on lysosomes.

Gražvydas Lukinavičius

"Biocompatible Probes for Imaging of Cellular Structures"

Department of NanoBiophotonics, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

The ideal fluorescent probe for bioimaging is bright, absorbs light at long wavelengths (> 600 nm) and can be flexibly implemented in living cells and *in vivo*. Typically, such probe consists of a fluorophore connected via a linker to a targeting moiety. The availability of targeting ligands is assured by a large number of studies aiming at the development of inhibitors for a wide range of biomolecules. However, the design of synthetic, highly biocompatible fluorophores has proven to be extremely difficult and is lagging behind. Recently, silicon-rhodamine was identified as a far red dye that can be specifically coupled to proteins, lipids and nucleic acids using different techniques. Importantly, its high permeability and fluorogenic character permit imaging of proteins in living cells and tissues, while its brightness and photostability make it ideally suited for live-cell super-resolution microscopy. Further investigations resulted in identification of cell-permeable fluorophores spanning the whole visible spectrum.

One of the most intriguing and challenging structures to image in the cell is chromatin. This biopolymer, composed of DNA and proteins, contains all information of the functional cell. I foresee the creation of probes highlighting chromatin by combining newly developed fluorophores and available chromatin-interacting small molecules. Super-resolution fluorescence microscopy has sufficient resolving power to provide information about spatial chromatin organization in living cells. Living cells imaging has the potential to reveal how the protein-DNA complex is organized during various cellular processes like cell division, stress or electrical stimulation.

Giulio Preta

"Studying Lipid Rafts: Six Degrees of Inner Turbulence"

GYŐZŐ SZENCI[1], Szabolcs Takáts[1,2], Gábor Glatz[1] , Attila Boda[1], Krisztina Hegedűs[1], Attila L. Kovács[1], Gabor Juhász[1,3]

Department of Bioelectrochemistry and Biospectroscopy, Life Sciences Center, Vilnius University, Vilnius, Lithuania

Lipids are essential building blocks of all cells and play an important role in the pathogenesis of different diseases, including cancer and neurodegeneration. Several studies have shown that lipids acquire specific organization in each cellular compartment and this organization is functional to the lipid function. For example, lipid microdomains in the plasma membrane, commonly known as lipid rafts, are functional platforms for protein receptors and can influence intracellular signalling. Because of the reduced size and the unstable nature of these membrane microdomains, the challenge for scientists is to develop advanced techniques to study their organization and function. Currently six main different techniques can be used, each of them presenting positive and negative aspects in terms of accuracy, easy of use and costs.

Diana Ikasalaitė

"In vitro repair of 5-alkylcytosines"

Department of Biological DNA Modifications , Life Sciences Center, Vilnius University, Vilnius, Lithuania

DNA methylation is found in both prokaryotes and eukaryotes where it plays important roles in various molecular mechanisms such as protection against viruses, regulation of gene expression, biological signaling, etc. Methylation is introduced by specific enzymes - DNA methyltransferases which catalyze the transfer of a methyl group from S-adenosyl-L-methionine to DNA. It is well known that in mammals DNA methylation occurs mostly at the 5th position of cytosine and acts as one of several epigenetic mechanisms. Despite its high abundance and importance, many methylation-related questions remain unanswered. Since this is partially due to methyl group being a poor reporter molecule, sophisticated techniques of tracking epigenetic cell programming must be developed. One of such novel techniques is called methyltransferase-Directed Transfer of Activated Groups, or mTAG. mTAG is a robust technique, which allows scientists to introduce extended reporter groups to DNA by covalent modification in a site-specific manner. It uses engineered methyltransferases and synthetic S-adenosyl-L-methionine analogues for sequence-specific DNA labeling and has proven to be a useful tool for optical DNA mapping, extraction of DNA from complex mixtures and epigenetic studies. For further studies it is necessary to find out how such modified DNA acts in a cell environment, particularly, if it is repaired by natural DNA repair systems. Therefore, the aim of this study was to examine whether 5-alkylcytosines, having namely ethyl-, propen-, butyn- or hexynazide- modifying groups (mTAG type or model modifications) are recognized and repaired by base excision repair enzymes. Since DNA glycosylases are the key enzymes in the base excision repair pathway, we tested multiple purified DNA glycosylases and found

that none of the enzymes used in this study show any activity on the modified bases in vitro. Therefore, we conclude that the tested 5-alkylcytosines are not repaired by base excision repair.

Gediminas Skvarnavičius

"Investigating Ligand Binding Induced Changes in Protein Volume using High Pressure Spectrofluorimetry"

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Introduction: The change in protein volume due to binding of a ligand (binding volume) opens a new dimension in parameters' space of protein-ligand interaction. However, this parameter is often neglected, mainly due to the laborious nature of experiments. Fundamental understanding of binding volume may lead to the new insights in the drug discovery field. Here we present a method that allows to determine binding volumes by exploiting ligand induced stabilization of proteins against pressure denaturation.

Aim: To develop and refine a method for accurate determination of binding volume.

Materials and methods: Systems of carbonic anhydrases, heat-shock protein 90 and their inhibitors were studied using a spectrofluorimeter with a high pressure system which sustains a hydrostatic pressure of up to 400 MPa. In certain cases protein destabilizing agents such as guanidine hydrochloride (GndHCl) were also used to reduce the energies of protein unfolding.

Results: Ligand binding resulted in stabilization of proteins against pressure denaturation. This change in melting pressures (Pm) was found to be proportional to the binding strength and concentration of the ligand. This allows calculation of the binding volume from a series of unfolding experiments which differ in ligand concentrations. In cases where achievable pressure was too low to obtain protein unfolding, GndHCl was used to lower the Pm of proteins. This dependence was found to be linear, therefore an extrapolation of Pm to a zero concentration of GndHCl was used to determine denaturant-free Pm. Stronger ligand binding showed more negative protein volume change, meaning that stronger ligands cause higher packing degree of a protein.

Conclusions: This study showed how high pressure spectrofluorimetry can be used to determine ligand binding volume. These volumes can be calculated from a series of pressure unfolding experiments using various ligand concentrations. Problems of unachievable melting pressures can be solved using denaturing agents.

POSTER PRESENTATIONS

Molecular and Cell Biology

Edina Brigitta Udvari

"The Insulin-like Growth Factor 1 (IGF-1) Treatment Cause Hypothalamus Protein Level Changes"

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Introduction: During motherhood, the behavioral and emotional states of females change robustly. These are also accompanied with molecular alterations. The hypothalamus is a main regulator of the appearance and maintenance of maternal behavior and lactation in the postpartum period. In previous studies, we identified IGF-1 as a common regulator of maternally altered hypothalamic proteins, and an increased maternal IGFBP-3 mRNA level in a microarray study. We also showed that intracerebroventricular injection of IGF-1 inhibited IGFBP-3 which led to increased pup retrieval time suggesting reduced maternal motivation.

Aim: To reveal the underlying molecular mechanisms the actions of IGF-1 in mother rats, we performed proteomic analysis on hypothalamic samples between IGF-1 treated and salinetreated control mother rats.

Materials and methods: We performed 2-D DIGE minimal stain technique and MS-based proteomic analysis on hypothalamic samples between IGF-1 treated and control maternal rats on postpartum day 14th. We validated the increased level of Dpysl2 protein with Western blot technique and we performed bioinformatic analysis on Pathway Studio Platform.

Results: We identified 26 significantly changed proteins, including 12 increased and 14 decreased proteins. Dyhidropyrimidinase-related protein 2 (Dpysl2), L-lactate dehydrogenase B chain (Ldhb) and Protein disulfide isomerase A3 (Pdia3) are the proteins with the highest fold changes. Aspartate aminotransferase (Got1), Septin-5 (Sept5) and Actin 2 (Actg1) are the three most decreased proteins.

Conclusion: The proteomic results support each other and give a better view of the underlying molecular mechanisms. Bioinformatical analysis revealed several connections between IGF-1 and common regulators of the changed proteins. We suggest that the IGF-1 effects may be achieved by these regulators. Few common regulators were present in both the decreased and increased proteins' regulators, which indicates their important regulatory roles.

Supported by the ÚNKP-17-3 New National Excellence Program of the Ministry of Human Capacities, KTIA-NAP_-13-2-2017-0007 Program and KTIA_NAP_13-2-2015-0003 Program.

Giedrė Karzaitė

"Fluorescence Microscopy Studies of DNA and DNA Restriction Enzymes Interactions at the Single Molecule Level"

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Restriction endonucleases (REases) recognize specific nucleotide sequences in a double stranded DNA and cleave both strands of the duplex. In vivo REases protect their host bacteria from viral attacks by cleaving foreign DNA. *In vitro*, they are widely used as a molecular tool for various DNA manipulations. Restrictase BfiI recognizes an asymmetric DNA sequence, 5'- ACTGGG-3', and cuts top and bottom strands at fixed positions downstream of this sequence.

Here we are studying several versions of BfiI REase: WT BfiI, BfiI-SS, BfiI-K107A, BfiI-K107A-SS. The wild-type BfiI is a native protein. In BfiI-SS protein SS bridge is linking the dimer interface. In BfiI-K107A protein mutation was introduced in the active center at residue 107 - Lysine was changed to Alanine. BfiI-K107A-SS protein contains both mentioned mutations.

In this research we have studied interaction between DNA and BfiI restriction enzymes using single – molecule Forster Resonance Energy Transfer (smFRET) total internal reflection (TIR) microscopy. It is a powerful method used for studying conformational dynamics and temporary inhomogeneous interactions. Biotinylated DNA

molecules bearing two targets for BfiI and FRET pair dyes close to these targets were immobilized on a silanized and PEGylated (metoxy-PEG and biotin PEG mixture) glass surface via neutravidin. The protein induces formation of DNA loop: one BfiI site binds one target and second another, and therefore brings the FRET pair in close proximity.

Our acquired smFRET signals have shown multiple FRET level lasting for several seconds. We observed that FRET efficiency and duration was affected by BfiI mutation, crosslinking and Mg^{2+} ions/ion strength.

Milda Norkienė

"Optimization of Polymavirus VP1 Protein Biosynthesis in *S. Cerevisae* Cells and Application of Recombinant Virus-like Particles for Serology"

Polyomaviruses (PyVs) represent quickly growing group of viruses found in mammals, birds, or fish. Twelve new human polyomaviruses (HPyVs) have been identified in past ten years. PyVs are small, non-enveloped viruses that contain double-stranded, circular DNA genome about 5000 base pairs in length. The icosahedral viral capsids of approximately 45nm in diameter is composed of three proteins, major VP1, and two minor: VP2, and VP3. When expressed in eukaryotic cells, VP1 proteins are able to self-assemble into virus-like particles (VLPs), which are used in assays to detect specific antibodies. In contrast to better-studied polyomaviruses JCPyV, BKPyV and partially MCPyV, the routes of infection, entry pathways and cell tropism of new HPyVs remain unknown.

HPyVs are part of the normal microbial flora and the infection of these viruses in their host is thought to be asymptomatic but, when host immunity is compromised the infection can cause acute systemic disease or tumor induction. These diseases include BK virus–related nephropathy, JC virus–related progressive multifocal leukoencephalopathy, trichodysplasia spinulosa, HPyV6 or HPyV7-related epithelial hyperplasia or Merkel cell carcinoma. Worldwide seroprevalence for HPyVs is reported to be approximately 80 % in adult individuals, as detected by anti-HPyV IgG antibodies (Abs) in human serum.

In order to generate high quality PyV VP1 VLPs, first task was to investigate some features of PyV VP1 protein synthesis in yeast *S. cerevisiae* cells. The impact of NLS in WUPyV VP1 VLPs yield and assembly quality were analyzed. The yeast expression system was successfully utilized for high-throughput production of recombinant VP1-derived VLPs from 11 newly identified HPyVs. HPyV12 VP1-derived VLPs were generated from the second of two potential translation initiation sites in the VP1-encoding ORF. Recombinant VLPs produced in yeast originated from different HPyVs demonstrated distinct hemagglutinating activities (in HA) and may be useful in virus diagnostics, capsid structure studies, or investigation of entry pathways and cell tropism of HPyVs until cell culture systems for new HPyVs are developed.

All purified HPyV VP1 VLPs were used to develop indirect enzyme immunoassay (EIA) for detection of HPyVs specific Abs in two groups of sera samples (1106 samples). We demonstrated that seroprevalence of HPyV was 18-67 % in the first group of serum samples collected in Lithuania and up to 30 % higher in second group of serum samples collected from high-risk persons venous blood in Bulgaria. The highest seropositivity determined in both serum sample groups was detected for WUPyV, MCPyV and TSPyV and confirmed results reported by some studies in other countries.

However, because of the high amino acid sequence identity between VP1 of some human polyomaviruses, cross-reactivity of antibodies is occasionally observed. In this study we found some degree of cross-reactivity between BKPyV and JCPyV, HPyV6 and HPyV7, HPyV9 and HPyV10, WUPyV and KIPyV VP1 VLPs. From 10 to 100 % anti-HPyV IgG antibodies in specimens of human serum samples were cross-reactive due to large number of different HPyV and VP1 sequence identity. It can be assumed that there has been evolutionary pressure for the immunogenic epitopes of the HPyVs to diverge so that several viruses can establish co-infections.

Giedrė Paužaitė

"Changes in Seed Germination, Hormone Content and Explant Growth Induced by Pre-Sowing Cold Plasma Treatment"

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The aims of study were to evaluate effects of atmospheric cold plasma (CP) irradiation on dynamics of radish (*Raphanus sativus*) seed germination, to estimate the content of main phytohormones involved in the control of germination, and to compare the development of plant explants derived from control and treated seeds. Freshly harvested radish seeds were treated for different durations (1, 3, 5, 7 and 10 min) with a scalable dielectric barrier discharge CP device.

Germination tests *in vitro* were performed and at the same time methanol extracts of dry seed homogenates were prepared for phytohormone analysis by HPLC. The indices of germination kinetics were determined using Richards plots. The effects on callus and adventitious root formation was assessed by culturing seed-derived *R. sativus* explants on the nutrient media.

CP treatment significantly increased V_i (%) - final germination percentage in all experimental groups of treated seeds and decreased M_e (days) - the median germination time, indicating that CP treatments stimulate germination. ABA content was strongly reduced (this change strongly correlated with stimulation of germination), and GA7 content was increased in in all groups of CP-treated seeds. Approximately, three-fold increases in the frequency of adventitious rooting in comparison to control were observed on dissected radish leaf segments developed from 1 min and 10 min CP treated seeds.

The obtained results demonstrate for the first time that stimulation of germination by CP treatment on the molecular level can be explained by the induced changes in the ratio of the main phytohormones (ABA and GAs) involved in the control of seed dormancy and germination, i.e., CP treatment works in the similar way as other seed dormancy breaking treatments. The obtained results indicate also potential of CP treatment for use in plant biotechnology.

Emilija Karazijaitė

"Construction and Characterization of the Deletion Mutant of Virulence Gene *ompA* in Clinical *Acinetobacter baumannii* Strain"

EMILIJA KARAZIJAITĖ, Jūratė Skerniškytė, Edita Sužiedėlienė

Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania

Acinetobacter baumannii is a Gram negative opportunistic pathogen which causes variety of nosocomial infections to immunosuppressed patients. Due to its ability to acquire multidrug resistance and persist in clinical environment *A. baumannii* is considered to be a global threat in health care settings. Several factors associated with *A. baumannii* virulence have been proposed. Special focus is given to bacterial surface properties as they are responsible for attachment to abiotic surfaces and to host cells. *A. baumannii* surface protein OmpA is β-barrel porin expressed in the outer membrane of bacteria. Due to high prevalence among *A. baumannii* strains and high expression on the bacterial surface, OmpA currently is one of the most promising vaccine candidates against this nosocomial pathogen.

The aim of this work was to access the importance of *ompA* gene for virulence properties exhibited by clinical *A. baumannii* strain.

For this purpose *A. baumannii ompA* gene knockout mutant was generated by using marker-less gene deletion technique based on a homologous recombination. For complementation experiments *ompA* gene with its native promoter was cloned into shuttle vector pUC19_gm_AcORI and resulting plasmid was used for transformation of Δ*ompA* strain. *A. baumannii* mutant and wild

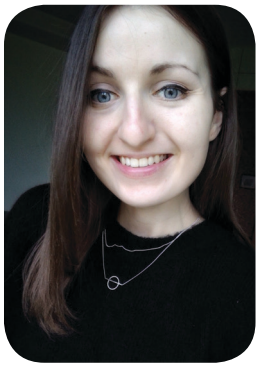
type strain were tested for ability to form biofilms using crystal violet staining. Serum bactericidal assay was performed as well.

According to our results, *ompA* gene deletion mutant was successfully generated in a clinical *A. baumannii* strain. *ompA* mutant showed reduced growth in liquid medium and serum, also impaired ability to form biofilm on polystyrene surface compared to the wild type strain. Complementation with *ompA* gene allele partly restored virulence properties of *ompA* deletion mutant.

To conclude, by generating deletion mutant and performing complementation experiments, we confirmed that *ompA* gene plays an important role in virulence-associated properties expressed by clinical *A. baumannii* strain.

Raminta Batiuškaitė

"Synthesis of Short Open Reading Frames of BACE1 mRNAs' Encoded Polypeptides in Yeast *Saccharomyces cerevisiae*"



RAMINTA BATIUŠKAITĖ, Rasa-Petraitytė Burneikienė, Kęstutis Sasnauskas

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Introduction: Alzheimer's disease (AD) is a progressive neurodegenerative disease which is the main cause of dementia worldwide. More than 46 million people are suffering from AD and at this moment there is still no approved effective treatment of this disease. AD is characterized by accumulation of β-amyloid peptide and hyperphosphorylated or truncated microtubule-associated tau protein in brains. Toxic β-amyloid peptide is cleaved from amyloid precursor protein (APP) by BACE1 secretase. It is

known, that BACE1 mRNAs' 5'UTR region contains three short open reading frames (sORFs), which might be associated with an increase of BACE1 secretase amount in AD cases, but it is still lack of information about these sORFs.

Aim: The aim of this study is to synthesize BACE1 mRNAs' 5'UTR sORFs encoded protein fragments in yeast *Saccharomyces cerevisiae* and assess if these recombinant chimeric proteins form virus-like particles (VLPs).

Materials and Methods: *BACE1* DNA coding fragments were inserted into yeast expression vector coding hamster polyomavirus *VP1* gene. The constructed vectors were verified by using *Escherichia coli* DH5α strain and after verification they were transformed into yeast *S. cerevisiae* 214 strain. After verification of chimeric proteins expression in yeast *S. cerevisiae*, these proteins were purified using ultracentrifugation through sacharose and cesium chloride gradient technique. Formation of VLPs was assessed using electronic microscopy. Recombinant chimeric VLPs proteins were transferred to the Department of Immunology and Cell Biology (Institute of Biotechnology, VU) for immunization of mice and generation of monoclonal antibodies.

Results and Conclusion: The expression vectors containing BACE, BACEG1, BACEG2 and BACEZ1 fragments were constructed successfully and the synthesis of the recombinant chimeric HAVP1-BACE, HAVP1-BACEG1, HAVP1-BACEG2 and HAVP1-BACEZ1 proteins in *Saccharomyces cerevisiae* cells was detected. Three of these chimeric proteins (HAVP1-BACE, HAVP1-BACEG1 and HAVP1-BACEZ1) formed VLPs.

Acknowledgments
This research was funded by a grant (No. SEN05/2015) from the Research Council of Lithuania.

Miglė Razgūnaitė

"Identification of *Mycoplasma* Spp. Pathogens in Domestic Cats in Lithuania"

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Introduction: *Mycoplasma haemofelis*, *Candidatus Mycoplasma haemominutum* and *Candidatus Mycoplasma turicensis* are minute, gram-negative, epicellular bacteria infecting the feline erythrocyte and causing immune-mediated hemolytic anemia (IMHA), thrombocytopenia, fever and jaundice. IMHA caused by *Mycoplasma* spp. pathogens may range from mild to severe disease in cats. Such difference in the virulence of *Mycoplasma* genus is associated with pathogenesis of different *Mycoplasma* genus, *M. haemofelis* being the most pathogenic. The infection is not limited to cats, and can be caught from or given to other companion animals. Humans are also at risk of infection. Definitive diagnosis of hemotropic *Mycoplasma* spp. infection is made by examination of a thin Wright-Giemsa-stained blood smear. However, examination under a microscope sometimes cannot detect the pathogens because the tiny organisms often resemble other blood artifacts.

Aim: The aim of the present study was to identify *Mycoplasma* species using molecular detection methods in cats from Lithuania.

Materials and methods: Blood samples were collected from 50 cats in pet clinics and animal shelters in Kaunas during 2016-2018. DNA was isolated from EDTA-anticoagulant whole blood.

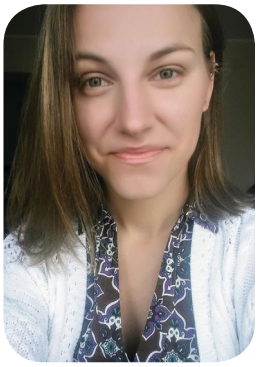
Detection of *Mycoplasma* was performed using Real-Time and conventional PCR targeting a 600-bp region of the 16S rRNA gene. PCR products were sequenced and then analyzed using BLAST and Mega software.

Results: Molecular analysis allowed detection of *Mycoplasma* DNA in 18% (9/50) of cats - 16% (3/18) in shelter cats and 18% (6/32) in cats brought to pet clinic. Sequence analysis of *Mycoplasma* isolates revealed the presence of two *Mycoplasma* species in cats – *Candidatus Mycoplasma haemominutum* and *Mycoplasma haemofelis.*

Conclusion: This study is the first report on molecular detection and characterization of *Mycoplasma* spp. in cats in Lithuania.

Paulina Amšiejūtė

"Molecular detection and characterization of *Bartonella* strains in small rodents"



PAULINA AMŠIEJŪTĖ, Dalytė Mardosaitė-Busaitienė, Jana Radzijevskaja, Algimantas Paulauskas

Faculty of Natural Sciences, Vytautas Magnus University, Kaunas, Lithuania

To date, 35 species in *Bartonella* genus have been officially recognized of which more than 15 *Bartonella* species have been isolated from small rodents. *Bartonella* infections in rodents and cases of human infections with *Bartonella* bacteria of rodent origin have been reported worldwide. However, there still is a lack of knowledge on the geographical distribution, genetic diversity and the reservoir hosts for these microorganisms in Baltic countries. This study aimed to investigate the prevalence and genetic diversity

of *Bartonella* spp. in small rodents in Lithuania. A total of 430 spleen samples obtained from wild-living small rodents of seven species were examined for the presence of *Bartonella* spp. by PCR. Species identification and molecular characterization of bacteria strains were based on sequence analysis of two housekeeping genes (*rpoB*, *groEL*) and the intergenic species region. *Bartonella* DNA was detected in 23.7% (102/430) of small rodents: in 33.2% of *Apodemus flavicollis*, 23.7% of *Micromys minutus*, 15.5% of *Myodes glareolus*, 12.5% of *Microtus oeconomus*, and in one of two specimens of *M. arvalis*. Sequence analysis of *Bartonella* isolates showed that the *Bartonella* strains circulating among the investigated rodents are heterogenic and belonged to *Bartonella grahamii*, *Bartonella taylorii* and *Bartonella rochalimae* genogroup. Phylogenetic analysis based on each of the targets demonstrated the presence of two different *B. grahamii* strains in *A. flavicollis*, *M. minutus*, *M. glareolus*, *M. arvalis*, *M. oeconomus*, and three *B. taylorii* strains in *A. flavicollis* and *M. glareolus*. *Bartonella* strain from *B. rochalimae* genogroup was detected in *M. glareolus*. This is the first report of the molecular characterization of *Bartonella* spp. in rodents in Lithuania. The frequent distribution of *Bartonella* species suggests that they may contribute to unidentified clinical infections. The impact of this bacterium on wildlife and human health needs to be determined.

Albinas Čepauskas

"Investigation of Acetylation Target of GNAT Superfamily Protein CheA from *Acinetobacter baumannii*"

ALBINAS ČEPAUSKAS, Julija Armalytė, Dukas Jurėnas, Edita Sužiedėlienė

Acinetobacter baumannii is a gram negative opportunistic pathogen which causes variety of nosocomial infections. Due to the ability to persist in clinical environment and rapidly acquire antibiotic resistant phenotype it has emerged as one of the most important human pathogens in hospital settings. Despite the increasing research efforts, factors which contribute to this successful emergence remain elusive. One of the factors could be toxin-antitoxin (TA) systems which are genetic loci abundant in low copy plasmids and chromosomes. They have been proposed to fulfil a myriad of roles in bacterial physiology, including plasmid stabilization, stress response, persistence and protection from phage infections.

In this work we set out to determine acetylation target of CheA protein, which was originally proposed to be an antitoxin of *A. baumannii* TA system CheTA. The system exhibits unique organisation, due to the toxin possessing an HTH fold, more common for the antitoxins, and antitoxin CheA possessing Gcn5 related N-acetyltransferase (GNAT) domain. To this day, in all characterized TA systems, GNAT domain possessing protein acts as a toxin and inhibits translation via N-acetylation of aminoacyl moiety of tRNAs, thus elucidation of CheA acetylation target would greatly aid in clarifying whether CheTA loci is in fact a novel TA system or just two independent genes mistakenly annotated as a TA.

To achieve this goal, *A. baumannii* extract, possibly containing the target has been purified via two sequential gel filtrations, followed by an HPLC assay. To identify the exact molecule we co-crystalised CheA with purified extract. Crystals yielded structure of CheA with additional electron density at the active centre, however for precise modelling of the target further MS analysis is required.

Even though we are yet to elucidate exact structure of the molecule acetylated by CheA, it is clear that it is not similar to targets of other GNAT proteins in TA systems. Further work is required to elucidate CheA acetylation target and confirm proteins role in the context of TA systems.

Inga Andriulė

"Non-homogeneous distribution of TRPM7 in human ventricular cardiomyocytes"

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2. Neuroscience Institute, Lithuanian University of Health Sciences, Kaunas, Lithuania

Introduction: TRPM7 (transient receptor potential melastanin 7) gene appears to be ubiquitously expressed, with highest expression in heart tissues, and participate in a variety of physiological/ pathological processes. However the direct evidence for the presence of TRPM7 proteins in human ventricular cardiomyocytes is still lacking.

Aim: To show the presence of TRPM7 protein in human ventricular cardiomyocytes, using immunofluorescence. Since the issue of

antibody specificity is a very critical one, for this reason we aimed to validate this procedure as much as possible with the different antibodies.

Materials and methods: We used enzymatically dissociated cardiomyocytes. After fixation, permeabilization and prevention of non-specific binding using blocking buffer, cells were incubated with primary mouse monoclonal or rabbit polyclonal anti TRPM7 antibody (1:200) in blocking buffer overnight at 4°C. For negative control, incubation with primary antibody was omitted. Further, the cells were incubated with fluorescently labelled secondary antibody AF488 (1:200), co stained with Phaloidin (1:100) and with Hoechst (25µg/mL), for labelling of the F actin cytoskeleton for contrast staining, and of the nucleus, respectively. Glass-slides were covered with Anti-fade Reagent.

Results: The confocal laser scanning microscopy images prove that TRPM7 can be detected on the surface membrane of human ventricular cardiomyocytes with both monoclonal and polyclonal anti TRPM7 antibodies. Data revealed that TRPM7 is inhomogeneously expressed in the plasma membrane, with higher expression at lateral or end to end inter-connections between single ventricular cardiomyocytes. No consistent difference was revealed between the staining of cells obtained from the same patient, when using different antibodies. In addition, there was no staining visible in negative control samples indicating that TRPM7 was labelled specifically by the antibodies.

Conclusion: TRPM7 protein is expressed in the plasma membrane of human ventricular cardiomyocytes, and the distribution is non-homogeneous.

Mark Bavirša

"DNA Methylation Studies in Renal Cell Cancer"

MARK BAVIRŠA[1], Kristina Daniūnaitė[1,2], Sonata Jarmalaitė[1,2]

1. Human Genome Research Group, Life Sciences Center, Vilnius University, Vilnius, Lithuania

2. National Cancer Institute, Vilnius, Lithuania

Introduction: Around half of renal cell cancer (RCC) cases are detected by accidental ultrasound imaging or other procedures. It is important to improve early diagnosis and to look for molecular biomarkers in order to facilitate both diagnosis of the disease and the selection of the most effective treatment regimen. Changes in the epigenetic landscape of RCC such as DNA hypermethylation could be a relevant tool for this task.

The **aim** of this study was to investigate the promoter DNA methylation status of selected protein coding genes as potential diagnostic biomarkers of RCC.

Methods: In total, 70 tissue samples (30 tumors, 10 peritumoral tissues, and 30 noncancerous tissues) obtained from 30 RCC cases were included in the present study. After review of published literature 6 candidate genes – *RARB*, *p16^{INK4a}*, *DACH1*, *PRKCB*, *FILIP1L*, and *NAALAD2* – were chosen for the methylation analysis. Fresh-frozen tissue samples were mechanically homogenized. DNA was extracted using a standard phenol-chloroform method. Bisulfite-converted genomic DNA was used for promoter methylation analysis by means of methylation-specific PCR.

Results: Promoter methylation frequency of *RARB*, *p16^{INK4a}*, *DACH1*, *PRKCB*, *FILIP1L*, and *NAALAD2* genes in tumors was 10%, 3%, 3%, 30%, 3%, and 47% respectively. No significant differences

in hypermethylation frequencies were observed between tumors and peritumoral or noncancerous tissues. Hypermethylation of *PRKCB* promoter was more frequent in tumors of advanced stage as compared to early stage (57% vs. 6%; P = 0.0043) and a similar tendency, although nonsignificant, was observed for *NAALAD2* (64% vs. 31%; P = 0.1413). No associations were observed between promoter methylation of the analyzed genes and tumor size, differentiation grade, and patients' age.

Conclusion: Our present study showed frequent *PRKCB* promoter hypermethylation in advanced RCC, which could be utilized for stratification of RCC at diagnosis. Further study in larger cohorts is needed to confirm our preliminary findings.

Kristina Žukauskaitė

"Promoter Methylation Analysis of *RARB*, *RASSF1* and *GSTP1* Genes in Prostate Cancer"

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1. Human Genome Research Group, Life Sciences Center, Vilnius University, Vilnius, Lithuania
2. National Cancer Institute, Vilnius, Lithuania

Prostate cancer (PCa) is the second most prevalent malignancy in men worldwide. Despite recent achievements in PCa diagnostics and treatment, new molecular biomarkers that could outperform the currently used ones are highly desirable. Aberrant DNA methylation in promoter region is considered as the earliest somatic genome change in cancer. Thus, promoter methylation of tumor suppressor genes could be used as biomarkers for PCa diagnostics and prognosis.

The aim of this study was to evaluate the promoter methylation status of tumor suppressor genes *RARB*, *RASSF1*, and *GSTP1* in clinical samples as potential PCa biomarkers.

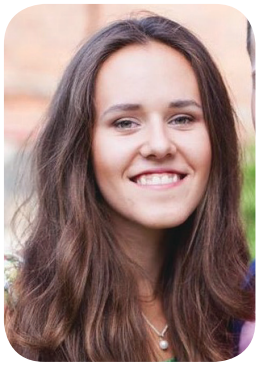
In the present study, 112 tumor samples and 16 noncancerous prostate tissues (NPT) from 113 PCa patients were analyzed by means of methylation-specific PCR (MSP). Also, DNA from voided urine samples of 44 PCa patients were tested using quantitative MSP.

Significantly different methylation frequencies of *RARB*, *RASSF1*, and *GSTP1* were observed comparing PCa and NPT samples (all $p < 0.0001$). The sensitivity of these biomarkers for PCa was up to 99%, while specificity reached 87%. Moreover, promoter methylation of *RARB*, *RASSF1*, and *GSTP1* was detectable in urine, where mean methylation levels comprised 0.02%, 0.04%, and 0.01%, respectively. In tumors, *GSTP1* was more frequently methylated in PCa cases with higher prostate mass ($p = 0.0112$). Methylation of all the three genes was also more commonly observed with increasing Gleason grade group, however, the associations were not statistically significant (all $p > 0.05$). No correlations were detected between gene promoter methylation and tumor stage, prostate-specific antigen level, or biochemical disease progression.

In conclusion, frequent PCa-specific promoter methylation of *RARB*, *RASSF1*, and *GSTP1* might serve as novel epigenetic biomarkers for PCa diagnostics, potentially applicable for noninvasive diagnostics. Adding *RARB*, *RASSF1*, and *GSTP1* promoter methylation status to currently used clinical tests has the potential to improve early PCa detection.

Justina Žvirblytė

"Measurement of Calcium Current in Bone Marrow Mesenchymal Stem Cells"



J. ŽVIRBLYTĖ[1], I. Uzielienė[2], E. Bernotienė[2], A.Alaburda[1]

1.Department of Neurobiology and Biophysics, Life Sciences Center, Vilnius University, Vilnius, Lithuania
2. Department of Regenerative Medicine, Centre for Innovative Medicine, Vilnius, Lithuania

Mesenchymal stem cells are multipotent stem cells that have an ability to differentiate into a variety of cell types, including osteoblasts, adipocytes and chondrocytes. This is basis for a number of emerging regenerative treatment strategies.

It is known, that calcium signaling is involved in stem cells ability to differentiate down the chondrogenic lineage, however the pathway of intracellular calcium concentration increase is not known. One possibility for calcium to get into the cell is through the voltage-gated calcium channels. Knowledge about specific calcium channels involved could provide extra tools for differentiation control, including activation of channels by electrical stimulation or manipulation of ion conductance by specific activators or blockers.

The presence of such channel subunits in membrane can be detected using molecular methods, yet the only way to prove that voltage-gated calcium channels are functional is by demonstrating the currents passing through them. This can be achieved using whole-cell patch clamp configuration, which enables a direct current measurement in living cell cultures.

Our main goal is to establish the technique of the whole-cell patch clamp recordings in order to characterize the calcium currents in undifferentiated mesenchymal stem cells and after differentiation into the chondrogenic lineage.

Nadežda Dreiže

"Proteomic Analysis of Breast Cancer Resistance against Anticancer Drug RH1 Reveals Importance of Cancer Stem Cells"

NADEŽDA DREIŽE[1], Dalius Kučiauskas[1], Marija Ger[1], Algirdas Kaupinis[1], Jonas Cicėnas[1, 2, 3], Mindaugas Valius[1]

1. Proteomics Center, Life Sciences Center, Vilnius University, Vilnius, Lithuania

2. Swiss Institute of Bioinformatics, Geneva, Switzerland

3. MAP Kinase Resource, Bern, Switzerland

The efficiency of current chemotherapeutic cancer treatment is often limited by acquired tumor resistance. The acquired resistance arises during treatment through various therapy-induced adaptive responses due to intrinsic tumor heterogeneity. Identifying biological processes and pathways that are crucial for drug resistance development provides potential targets for successful combinatorial treatment.

The aim of this study is to investigate mechanism of action and acquired resistance of a novel anticancer agent RH1 designed to be enzymatically activated by NQO1 in cancer cells in the NQO1-independent way.

Global differential proteome, kinome and phosphoproteome analysis shows that RH1-resistant cells develop changes in their cell cycle progression, increase in anti-apoptotic signaling and are enriched with cancer stem cells. Moreover, stem cell factor and its receptor c-KIT autocrine signaling contribute to stemness and can serve as secondary target in treating RH1-resistant breast cancer.

This demonstrates that RH1 can be useful to treat cancers in NQO1-independent way and predicts that targeting of cancer stem cells might be effective approach combating cancer resistance to the RH1.

Laurita Klimkaitė

"Functional Screening of Antibiotic Resistance Genes in Soil *Chryseobacterium* spp. Genomic DNA Libraries"

LAURITA KLIMKAITĖ, Renatas Krasauskas, Julija Armalytė, Modestas Ružauskas, Edita Sužiedelienė

Background: Species of the genus *Chryseobacterium* are abundantly found in soil and water and have recently emerged as opportunistic nosocomial pathogens in humans. Due to multidrug-resistant phenotype, displayed by some species, and well documented examples of gene exchange between the environmental and the clinical strains, the more detailed picture of *Chryseobacterium* resistance mechanisms is needed. Therefore, functional genomic screening was applied in order to identify antibiotic resistance determinants in environmental *Chryseobacterium* spp. isolates.

Materials and Methods: Functional screening of *Chryseobacterium* spp. genomic DNA library from soil isolates were carried out with clinically important antibacterial agents which include: aminoglycosides (gentamicin, kanamycin, streptomycin), beta-lactam antibiotics (imipenem, cefuroxime), tetracycline, chloramfenicol, benzalkonium chloride and ciprofloxacin. Minimum inhibitory concentrations of clones were determined and interpreted according to EUCAST breakpoints. The inserts displaying resistance phenotype were sequenced and analyzed using bioinformatic approach.

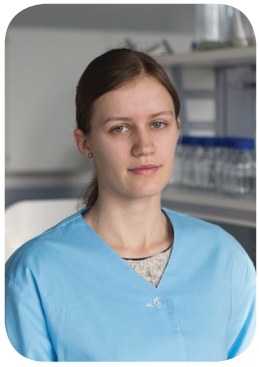
Results: Selections with streptomycin, tetracycline and cefuroxime resulted in single resistant clone per antibiotic. Two resistant clones were identified using imipenem and the same resistant clone was identified in separate selections with kanamycin or

gentamycin. Analysis of sequences revealed that in 4 out of 6 inserts contained genes coding directly antibiotic modifying enzymes-streptomycin adenylyltransferase, tetracycline MFS transporter, metallo-like beta-lactamase and carbapenem-hydrolyzing IND beta-lactamase. The remaining inserts did not contain obvious resistance genes. Resistance to imipenem may be determined by the GNAT family N-acetyltransferase or monooxygenase, while the lipid A biosynthesis acyltransferase may be responsible for the resistance to gentamycin and kanamycin.

Conclusion: The search for resistance determinants in soil *Chryseobacterium* spp. genomic libraries has found genes coding antibiotic modifying enzymes and genes not directly associated with resistance. Presence of clinically important resistance genes in soil *Chryseobacterium* may indicate significant antimicrobial pressure in environment and possibility of soil resistance mechanisms to be transferred to clinical settings and *vice versa.*

Raminta Mineikaitė, Gabrielė Trečiokaitė and Sigita Grigaitytė

"The Landscape of Small non-coding RNAs in Probiotics *L. casei* and *L.lactis*"



RAMINTA MINEIKAITĖ[1], GABRIELĖ TREČIOKAITĖ[1], SIGITA GRIGAITYTĖ[1], Milda Mickutė[1], Kotryna Kvederavičiūtė[2], Giedrius Vilkaitis[1]

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Introduction: RNA-mediated gene expression regulation is found in all kingdoms of life. Even though until recently only little was known about bacterial small non-coding RNAs (sRNAs), these 40-500 nt molecules are now considered as major post-transcriptional regulators of gene expression in bacteria. This study covers identification and analysis of novel sRNAs possibly responsible for regulation of cell wall formation in lactic acid bacteria (LAB) *Lactococcus lactis*, *Lactobacillus casei* widely used in food production and medicine.

Aim: To reveal *L. lactis* and *L. casei* sRNA transcriptomes and identify sRNAs responsible for stress mediated cell wall formation.

Materials and methods: RNA was extracted from *L. casei*, *L. lactis* in different growth phases. We designed a new protocol for 50-500 nt LAB sRNA library preparation for *Illumina*™ sequencing. To identify differentially expressed L. lactis sRNAs, sRNA libraries were prepared after the bacteria exposure to cell wall targeting antimicrobials (lysozyme or penicillin G) or growth in optimal medium. The potential sRNAs were experimentally confirmed by Northern hybridization.

Results: ~300 *L. casei* and ~240 *L. lactis* putative sRNAs were identified in sequencing data. 27 of 30 and 16 of 22 selected newly identified sRNAs in *L. casei* and *L. lactis*, respectively, were experimentally verified. Three of *L. lactis* sRNAs differentially expressed in response to lysozyme or penicillin G affect bacteria resistance to antimicrobials and potentially regulate cell wall biosynthesis.

Conclusion: sRNA transcriptome analysis uncovered hundreds of potential sRNA genes in the genome of *L. casei* and *L. lactis.* These results provide an excellent basis for further investigations on the molecular biology of lactic acid bacteria since some of the identified bacterial sRNA may have a major role in bacterial cell wall formation.

The work was supported by a grant from the Research Council of Lithuania MIP-059/2015

Rita Banciul

"*APC* Gene Promoter Methylation in Breast Cancer"

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2. Human Genome Research Group, Life Sciences Center, Vilnius University, Vilnius, Lithuania
3. National Cancer Institute, Vilnius, Lithuania

Introduction: Breast cancer is the most common oncological disease among women in Lithuania and many western countries. Since early diagnosis increases the chance of survival, it is of high importance to search for biomarkers, which would enable clinicians to detect breast cancer at its early stages. DNA methylation

biomarker is defined as a molecular target that undergoes DNA methylation changes in carcinogenesis. Aberrant DNA methylation in the promoter regions is commonly associated with silencing of tumor suppressor genes. Such epigenetic biomarkers might be utilized for early diagnosis of cancer, predicting and/or monitoring the therapeutic response.

The **aim** of this study was to evaluate the promoter methylation of the tumor suppressor gene *APC*, as a potential biomarker for breast cancer diagnosis.

Materials and methods: 74 breast carcinomas and 29 control samples were analyzed for *APC* promoter methylation. First, bisulfite conversion was used to convert unmethylated cytosine to uracil. Methylation specific PCR (MSP), which uses different primers for methylated and unmethylated DNA, was used in order to qualitatively determine the methylation status. MSP products were visualized by agarose gel electrophoresis.

Results: Methylation frequency of *APC* was 54.6% and 0% in breast tumors and control samples, respectively ($p < 0.0001$), showing its high specificity for cancer. APC methylation was also analyzed according to clinical-pathological patients' parameters. Statistically significant difference in methylation frequencies was found between luminal A (61%) and triple negative (18%) subtypes of breast cancer, which are the two most distinct molecular subtypes. No associations were found between *APC* methylation status and patient's age, tumor stage, tumor size, or disease recurrence/progression.

Conclusion: Our study revealed that *APC* gene promoter methylation could be utilized for diagnostics of breast cancer. However, further validation in independent cohort is needed.

Ernesta Pocevičiūtė

"Interaction of Biomolecules Using FRET and TIRF Microscopy"

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One of the most important areas in pharmacy, medicine and biotechnology is interacting molecules research, which allow to establish more effective medicines for disease treatment. This involve for cell-cell communication important receptors, cell adhesion molecules, channels ant transporters. These days single molecule observation methods enable us to investigate molecules interaction characteristics on the cell surface more accurately. In this research, we choose to investigate granulocyte colony-stimulating factor receptor (GCSFR) as a model protein, which normally in the cells surface interact with its ligand – GCSFR. Ligand connection to receptor induce receptor monomers dimerization and then activating signal transmission in cell. Applying SNAP-tag technology, when fused target proteins monomers with SNAP protein are labelled with different probes we can observe fluorescence resonance energy transfer (FRET) signal. By using total internal reflection fluorescence (TIRF) microscopy when labelled monomers get closer we obtain apparent FRET signal. Following signal intensity extensive purity of time, it is possible to evaluate the distance between two interacting receptor monomers and the duration of this interaction. Likewise operating with GCSFR ligand using the same method we may get more accurate information about receptor and its ligand connection time and variation in time, distance between receptor monomers when ligand is attached.

Lina Kačenauskaitė

"Investigation of Carbonic Anhydrase Knockout HeLa Cell Line"

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Introduction: Cancer remains a leading cause of death worldwide and despite major achievements in therapy there is a high need for developing new diagnosis and treatment strategies. We are interested in targeting specific proteins that are crucial for cancer cells. One of these targets is cabonic anhydrase IX (CA IX). CA IX is present in few normal tissues, but its ectopic expression is strongly associated with many frequently occurring tumours. The CA IX enzyme plays an important role in maintaining tumor cell homeostasis while acidifying the tumor microenvironment, which is a sign of poor prognosis. Consequently, chemical inhibitors are being investigated for targeted therapy. In order to investigate CA IX impact on cancer cells and inhibition efficiency of CA IX, a CA IX knockout HeLa cell line was generated using CRISPR/Cas9 system.

Aim: The aim of this research was to investigate the generated CA IX knockout HeLa cell line and compare with WT HeLa cell line as well as generate constructs containing resistance for puromycin gene for more efficient CA IX gene knockout in MHC-7 cells using CRISPR/Cas9 system. CA IX knockout cancer cell lines might benefit in advancement of CA IX function analysis in cancer cells and CA IX inhibition essays.

Materials and methods: Western blot analysis was performed in order to investigate CA IX knockout HeLa cell line. Various cloning methods were applied during CRISPR/Cas9 constructs generation.

Results: It was shown that in HeLa CA IX knockout cell line CA IX is absent on protein level. Moreover, it was noticed that loss of CA IX function is compensated in tumor cells. Therefore, another CA isoform associated with cancer, CA XII expression was investigated. Western blot analysis showed no significant changes of CA XII expression in HeLa CA IX knockout cells. Furthermore, three constructs containing gRNA for CA IX knockout and puromycin resistance gene were successfully obtained and are now being transfected to MHC-7 cells.

Conclusion: CA IX knockout cell lines are useful tools in loss-of-function research. It helps to understand CA IX role in cancer and serves as a negative control in CA IX inhibition assays.

Dovilė Tamoliūnaitė

"Identification of Babesia canis Genotypes in Dogs from Lithuania"

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Canine babesiosis is a widespread tick-borne disease caused by haematozoan parasites of the genus *Babesia*. The vast majority of clinical babesiosis cases in dogs in Europe is caused by *Babesia canis*. Canine babesiosis has become quite frequent in Lithuania during the past decade. Babesiosis caused by *B. canis* may range from mild to severe disease in dogs. Such difference in the virulence

of *B. canis* strains is associated with genetic heterogeneity among *B. canis* strains.

We aimed to investigate the genetic diversity of *B. canis* strains isolated from naturally infected dogs in Lithuania using PCR-RFLP assay and sequence analysis based on 18S rRNR gene.

In total 138 blood samples from dogs suspected of babesiosis were collected in Lithuania during 2016-2017. DNA was isolated from EDTA-anticoagulated whole blood. A partial region of the 18S rRNA gene of *B. canis* was amplified through PCR. PCR products were submitted to digestion with *HincII* restriction enzyme and evaluated by agarose gel electrophoresis. PCR products of 18S rRNA gene were sequenced and then analyzed using the Mega software.

According to PCR-RFLP analysis two genotypes of *B. canis* were identified: 97.9 % samples contained 18S RNA-A genotype, while 2.02 % – 18S rRNA-B genotype. Three 18S rRNA genotypes of *B. canis* were distinguished on the basis on two nucleotide (GA-> AG) substitutions observed in 18S rRNA gene sequences: 18S rRNR-A, 18S rRNR-B and both 18S rRNR-A/18S rRNR-B.

The molecular analysis indicates the presence of three *B. canis* genotypes in Lithuania, with the dominance of less virulent genotype against more virulent in tested samples. The results of the present study show the necessity to study the relationship between the genetic structure of *B. canis*, their geographical distribution, and the form of the disease in dogs.

This research is funded by the European Social Fund under the No. 09.3.3-LMT-K-712-03-0081 "Development of Competences of Scientists, other Researchers and Students through Practical Research Activities" measure.

Gabija Goptaitytė

"Detection of Antibiotic Resistance Determinants in Bacteria Isolated from Fish"

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Introduction: For decades antibiotic therapy was the most efficient treatment of infectious diseases. However, microbes reacted to antimicrobial agents by developing antibiotics resistance (AR). Spreading of multidrug-resistant bacteria is a worldwide problem. The clinically relevant bacterial AR genes are constantly spreading to the environment from human and animal sources. Specific DNA elements integrons enable the spreading of AR genes between different bacterial species.

Aim: The aim of this study was to examine the prevalence of genetic determinants responsible for antibiotic resistance in bacteria isolated from wild and farmed fish.

Materials and methods: A total of 115 bacterial isolates from fish obtained from fish farming (95) and natural waters (20) were examined for the genes conferring resistance to clinically important antibiotics (aminoglycosides, β-lactams, fluoroquinolones, chloramphenicol, tetracyclines, macrolides, glycopeptides), biocides and for the carriage of class 1 and 2 integrons. Detection has been performed using PCR with specific primers, integron structure was accessed by DNA sequencing and bioinformatic analysis.

Results: Genes conferring resistance to aminoglycosides (*aph (6) Id, ant (3 ,') Ib, aac (3) IIa*), β-lactams including 3th generation of cephalosporins (*oxa1, ctx-M*), fluoroquinolones (*qnrS*) and biocides (*qacE*) were found. Bacterial isolates from fish obtained in natural water pond and river were distinguished by multiple antibiotic resistance profile, whereas bacteria isolated from fish obtained in breeding farms and supermarkets harbored genes responsible for biocide resistance. Integrons were rare and most of them carried no gene cassettes. Class 1 integron with integrated gene cassettes was found in two bacterial isolates from wild fish (Nemunas river). Integrons carried aminoglycoside adenylyltransferase *aadA2* gene and dihydrofolate reductase *dfrA12* gene responsible for streptomycin and trimethoprim resistance, respectively.

Conclusion: Bacterial isolates from wild fish harbour substantial diversity of genes, conferring resistance to clinically important antibiotics and therefore present a potential source of contamination with AR bacteria.

Simona Sutkuvienė

"Synthesis of Carbazole-Based Derivatives as Potential Efflux Pump Inhibitors"

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Antimicrobial resistance is a worldwide problem in human and veterinary medicine. It occurs when micro-organisms such as bacteria, fungi, viruses, and parasites evolve resistance to antimicrobial substances, like antibiotics, antifungals and others. This occurs naturally during adaptation to the environment or when microorganisms come into contact with antimicrobials. The appearance of multiple resistant bacteria of human and veterinary origin is accelerated due to the inappropriate or excessive use of antimicrobials. As a result, medicines that were once effective in people and animals become less effective or not effective at all, leading to a reduced ability to treat infections. It seems that antibiotic resistance is inevitable. Therefore many different solutions have been proposed to solve this problem. As the ability to pump antibiotics out of cells is a common feature of the microbes and their pathogenic relatives and is the most widespread form of resistance to most classes of antibiotics that is why it is very important to discover molecules inhibiting efflux pumps as well as to reveal the inhibition mechanisms. There is another possibility to investigate similar substances as competitors of antibiotics.

Derivatives of carbazole could be considered as potential efflux pump inhibitors or substrates. Carbazole is heterocyclic compound

with two benzene rings linked in a tricyclic system through nitrogen atom and also could be employed as biological active compound. Such derivatives could be employed as antibacterial, antiviral, anti-inflammatory, anticancer, sedatives or tranquilizers agents. The slight change in the structure of these compounds causes distinguishable difference in their biological activities.

In this work we have synthesized carbazole-based molecules by one step synthesis. Synthesis paths, purification and structure identification methods will be presented. To determine the susceptibility of the synthesized compounds to the bacteria, to study their interaction with efflux pumps in *Salmonella enterica* bacteria it is planned in the future research step.

Raminta Mazėtytė

"Impact of Polypyrrole on Fluorescence Quenching of Glucose Oxidase"

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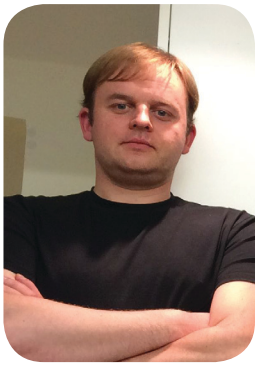
The active part of the glucose biosensor is a glucose oxidase (GOx) enzyme immobilized on the surface of the electrode. Immobilization of GOx is a very important issue affecting the enzymatic activity, biodegradability, longevity and stability, which is one the most important and challenging characteristics in practical application of biosensors. The purpose of this research was to evaluate impact of polypyrrole (Ppy) composites on stability of GOx enzyme.

Spectroscopic properties of GOx, flavin adenine dinucleotide (FAD) and composites of these compounds with Ppy were investigated in a sodium acetate buffer solution. In the present study, the nanosecond fluorescence quenching was observed for GOx/Ppy and FAD/Ppy composites by the steady-state absorption and time-resolved fluorescence spectroscopy technique.

The experimental data analysis showed that the Ppy, which formed composites with FAD and GOx, facilitated the removal of FAD molecules from GOx and twice reduced the fluorescence decay rate. Differences of the FAD and Ppy average fluorescence relaxation times showed that the FAD composite with Ppy (in such a way) so that the Ppy effectively quenched the FAD fluorescence and FAD could not freely unfold. During this study, it was found that GOx coated with Ppy is more stable compared to free GOx dissolved in solution, because non-encapsulated GOx dissociates to FAD and apo-enzyme.

Sigitas Palikša

"Decreased Km to dNTP's is an essential M-MuLV reverse transcriptase adoption required to perform efficient cDNA synthesis in one-step RT-PCR assay"



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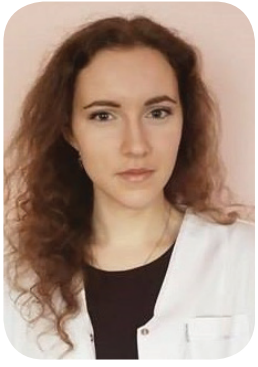
Personalized medicine and advanced diagnostic tools based on RNA analysis are focusing on fast and direct one-step RT-PCR assays. First strand cDNA synthesized by the reverse transcriptase is exponentially amplified in the end-point or real-time PCR. Even a

minor discrepancy in PCR conditions would result in big deviations during the data analysis. Thus, one-step RT-PCR composition is typically based on the PCR buffer. In this study we have used compartmentalized ribosome display (CRD) technique for in vitro evolution of the M-MuLV reverse transcriptase that would be able to perform efficient full-length cDNA synthesis in PCR buffer optimized for Taq DNA polymerase. The most frequent mutations found in a selected library were analyzed. Aside from the mutations, which switch off RNase H activity of reverse transcriptase and are beneficial for the full-length cDNA synthesis, we have identified several mutations in the active center of the enzyme (Q221R, V223A/M), which result in 4-to-5-fold decrease of Km for dNTPs (<0.2 mM). The selected mutations are in surprising agreement with the natural evolution process because they transformed the active center from the oncoretroviral M-MuLV RT-type to the lenitiviral enzyme-type. We believe that this was the major and essential phenotypic adjustment required to perform fast and efficient cDNA synthesis in PCR buffer at the 0.2 mM concentration of each dNTP.

Aistė Imbrasaitė

"Epitope Mapping of Monoclonal Antibodies Against Human Carbonic Anhydrase XII"



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Introduction: Human carbonic anhydrase XII (CA XII) is a transmembrane protein that catalyzes the reversible hydration of carbon dioxide and is being recognized as a potential biomarker

for different tumors. Monoclonal antibodies (MAbs) are widely used as diagnostic and therapeutic reagents in oncology. An important step in the characterization of MAbs, especially those used in therapeutic strategies, is the identification of the binding sites (epitopes) of these antibodies on their target antigens.

Aim: We aimed to construct overlapping CA XII protein fragments to identify the epitopes of MAbs raised against recombinant CA XII.

Materials and methods: MAbs against CA XII were generated by hybridoma technology and characterized previously. The full-length extracellular domain of CA XII and 8 hexahistidine-fused overlapping fragments (fragment # 1 (aa 1–94), fragment # 2 (aa 41–175), fragment # 3 (aa 166–264), fragment # 4 (aa 75–264), fragment # 5 (aa 9–195), fragment # 6 (aa 17–184), fragment # 7 (aa 24–207) and fragment # 8 (aa 33–196)) of CA XII were expressed in E. coli. The reactivity of the MAbs with CA XII extracellular domain and CA XII fragments was analysed by Western blot.

Results: In total, 10 MAbs (1D5, 4A6, 5D2, 8C9, 9A8, 13F5, 15A4, 6G5, 14D6, 20G7) recognized the extracellular domain of CA XII protein by Western blot. Specific binding of the MAbs with the constructed CA XII fragments was observed. The accurate aa sequence of the epitope recognized by the MAb 14D6 was determined. It was shown, that the MAb 14D6 binds near the active center of CA XII, which explains the inhibitory properties of this antibody.

Conclusion: This study provides new data on the CA XII-specific MAbs, that are promising diagnostic and therapeutic tools which can be applied for the diagnostics and therapy of various types of cancers.

Aistė Kveselytė, Karolina Dzedulionytė

"Synthesis of Novel Heterocyclic Amino Acids Possesing 1,3-Selenazole Structural Unit"

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Conjugation of chiral cyclic amino acids with heteroaromatic carboxylic acids can provide an opportunity for developing novel, conformationally constrained analogues of biologically important amino acids. When both the amino and carboxylic acid functional groups of this amino acid are employed in the construction of a molecular skeleton, the formation of a chiral condensed heterocyclic system can be achieved. (S)-Pyrrolidinyl-2-carboxylic acid (L-proline) is an important scaffold for the preparation of various functionalized heterocycles.

In the present work L-proline and related heterocyclic amino acids were used in the preparation of novel methyl 2-amino-1,3-selenazole-5-carboxylate derivatives possessing a chiral N-Boc-protected cycloaminyl substituent on their heteroaromatic ring. A series of methyl 2-amino-1,3-selenazole-5-carboxylates possessing a chiral pyrrolidin-2-yl, piperidin-2-yl or piperidin-3-yl substituent at C-4 of the heteroaromatic ring were designed and synthesized. The structures of the novel heterocyclic compounds

were confirmed by ^{1}H, ^{13}C and ^{77}Se NMR spectroscopy and HRMS investigations.

The obtained chiral amino acids can potentially be used as scaffolds for the synthesis of more complex molecules with biological activity or be used as building blocks for the development of DNA-encoded chemical libraries where peptide bond formation is necessary.
This work has been accomplished with financial support provided by Vipergen ApS corporation (Copenhagen, Denmark).

Justinas Babinskas

"Investigation of Laccase Substrate Oxidation at T1 Cu Site by Quantum Chemistry Methods"

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Laccases are multi-copper oxidases (EC 1.10.3.2), containing T1, T2 and T3 copper sites. Their natural substrates are phenolic and aromatic primary and secondary amine compounds. A lot of information is already accumulated throughout the years of their analysis (redox potentials, enzyme sources, reorganization energy, etc.). Laccases transfer one electron and proton from substrate to T1 Cu (oxidation); transfer one electron from T1 to T2/T3 Cu cluster; the T2/T3 cluster reduces one oxygen molecule to two water molecules, by using four electrons and protons. However, from the aforementioned phases of catalysis mechanism, not a single one of them is fully understood.

With this project, we present the most probable progression of T1 Cu – substrate oxidation phase, produced by quantum chemistry methods.

For this work, we used *ab initio* methods: i) Restricted Open-shell Hartree-Fock (ROHF) ii) ROHF with Møller-Plesset Perturbation Theory second level correlation correction (MP2) iii) Density Functional Theory (DFT), B3LYP and M06-L functionals. These methods were used with STO-3G, 3-21G, 6-31G, TZV, cc-pVDZ and cc-pVTZ basis sets. T1 Cu site was modeled after *Bacillus subtilis* laccase T1 Cu (PDB file 1GSK), by interchanging two histidines with two imidazoles, cysteine with CH_3-S- fragment. Axial ligand was excluded as it can vary depending on laccase source or mutation. Substrates were phenol, benzyl alcohol and 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO).

The most notable result of this work was the transition state of the oxidation phase. It gave intricate data of:

- simultaneous electron and proton transfer;
- conjugation between substrate and the T1 complex;
- difference between investigated substrates.

Our computed model's results are in conjunction with experimental data and even provide the information, which is unattainable or difficult to get experimentally; furthermore, it can be used to explore some of the reaction characteristics (activation energy, bonds lengths, *etc*.).

Gytis Druteika

"The Impact of E100K, F154V and V174I Mutations on the Tertiary Structure and CatalyticActivity of GD-95 Lipase"

GYTIS DRUTEIKA, Audrius Gegeckas, Eglė Lastauskienė, Renata Gudiukaitė

Introduction: The enzyme engineering and analysis *in silico* by various bioinformatic methods are powerful tools for better understanding of relationships between the structure and function of target proteins including lipolytic biocatalysts. In a previous work the new GD-95RM lipase with improved properties was designed through random mutagenesis. This enzyme showed higher catalytic activity and ability to catalyze reactions at higher temperatures compared to parental GD- 95 lipolytic enzyme.

Aim: During this study it was aimed to investigate the impact of obtained E100K, F154V and V174I mutations on the structure of GD-95 lipase.

Materials and methods: The analysis *in silico* using CAD-score web server and JMol software was carried out to determine how the distances between amino acids, responsible for *Geobacillus* lipase functionality and activity are related to the varying industrially important physicochemical characteristics (optimal temperature, pH, substrate affinity, etc.) in parental and mutated enzymes.

Results: The analysis *in silico* demonstrated that E100K, F154V and V174I mutations have significant influence on the distance between catalytic amino acids and/or amino acids involved into the substrate binding channel. Our results showed that after mutations the distance between catalytic Ser113 and His358 increased (4.78 Å in GD-95RM instead 3.79 Å in parental GD-95 lipase). Meanwhile,

the distance between Ser113 and Asp317 became shorter (7.04 Å compared to 7.82 Å). Newly formed or lost interactions by Phe16 or other amino acids, involved in the substrate binding channel may have caused the improvement of industrially–attractive characteristics.

Conclusion: This study showed that E100K, F154V and V174I mutations changed the structure of parental GD-95 lipase leading to production of new *Geobacillus* lipase variant GD-95RM with improved catalytic activity. The design and selection of new enzyme variants may help understand the structure-function relationships of biocatalysts and widen the known information about genotype-phenotype space of *Geobacillus* lipases.

Gita Matulevičiūtė

"Synthesis of (Het)arylquinazolin-4(3H)-one Derivatives as Potential Antioxidant"

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Introduction: Quinazolinone ring system is a significant structural unit of naturally occurring or synthetically produced biologically active compounds. Quinazolin-4(3H)-one derivatives exhibit a wide range of biological activities such as antibacterial, antifungal, antiviral and antioxidant. Examples can include some well-known drugs. Diproqualone which exhibits sedative and analgesic properties or Afloqualone which is known for its muscle-relaxant effects.

Aim: The aim of our work is to develop methods for the synthesis of analogues of 2-(het)arylquinazolin-4(3H)-one and investigate biological activities of its derivatives.

Materials and methods: The required for the synthesis 2-thioxo-2,3-dihydroquinazolin-4(1H)-one were obtained from the methyl anthranilate using several synthesis steps. 2-(Het) arylquinazolin-4(3H)-ones can be directly obtained from 2-thioxo-2,3dihydroquinazolin-4(1H)one by Pd-catalysed *Stille*-type CuBr*Me2S promoted Liebeskind-Srogl reactions with (het) arylstannanes or by a two-step procedure via their S-benzylated derivatives applying CuMeSal promoted *Suzuki*-type Liebeskind-Srogl reactions with arylboronic acids.

DPPH and ABTS free radical scavenging methods are usually applied to estimate antioxidant properties of examined compounds. Reduction of violet DPPH radical colored solution to corresponding hydrazine is obtained by receiving a hydrogen atom from antioxidant. The absorption band of resulting decolorization is registered at 517 nm. Blue-green ABTS radical colored solution is reduced by hydrogen-donating antioxidant and an absorption is measured around 734 nm.

Results: 2-(Thiophen-3-yl)quinazolin-4(3H)-one, 2-(thiophen-2-yl)quinazolin-4(3H)-one and 2-(naphthalen-2-yl)quinazolin-4(3H)-one showed the best biological abilities, especially 2-(thiophen-2-yl)quinazolin-4(3H)-one.

Conclusion: The obtained compounds possessed ortho-positioned onto the pyrimidone ring might be promising for the monitoring of diseases, particularly those associated with free radical scavenging.

Mantas Baliukynas

"Novel Method for Determination of Esterase Activity"

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The demand of enzymes in industry is increasing constantly due to their peculiar properties such as regio-, stereospecificity and biodegradability. According to the type of catalyzed reaction, enzymes are devided into six classes: oxidoreductases, transferases, hydrolases, lyases, ligases, isomerases. Hydrolases are the most explored and employed enzymes – the use of them covers almost 60 % of industrial enzyme market. However, the development of rapid and easy handling activity assays for hydrolases in research and industrial processes still faces the challenges. The majority of hydrolases is esterases. A large number of esterase activity assays have been investigated during recent years which are based on measuring the amount of released p-nitrophenol which is the result of enzymatic hydrolysis of p-nitrophenyl derivatives. One of the most common used substrates is p-nitrophenylacetate; however, this substrate has the abillity to decompose spontaneously by the autolysis. Another class of substrates based on indoxyl derivative is stable, and moreover, could be used for activity screening assays on agar-agar plates.

By this study we were aiming to develop esterase activity method using indoxyl acetate as the substrate. This method is based on colour changes at a wavelength of 375 nm. The main parametres for activity assay were estimated such as the dependance of reaction rate on different buffers, the concentration of substrate and enzyme, temperature, pH, etc. The results will be more presented in detail during the poster session.

Greta Musteikytė

"Methylene Blue Acts as Amyloid Remodelling Agent on Superoxide Dismutase I Aggregation"

GRETA MUSTEIKYTĖ, Vytautas Smirnovas

Introduction: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that results in motor neuron death and has no approved treatment yet. Around 20% of ALS cases are caused by Cu, Zn human superoxide dismutase (SOD1) aggregation into amyloid fibrils. First step of potential strategies to combat the disease could be finding an effective aggregation inhibitor *in vitro*. However, only a few studies for SOD1 protein have been done in this field for now. They involve *in vitro* screening of 640 FDA approved drugs, several flavonoids and curcumin only.

Aim: Our goal was to test inhibitory effect of a small molecule methylene blue, which has been shown to inhibit Alzheimer's disease-linked amyloid beta oligomerization.

Materials and methods: Recombinant SOD1 was purified from *E. Coli* by Ni^{2+} affinity chromatography. Aggregation experiments were carried out in 10 mM potassium phosphate, 0.5 M GuHCl and 5 mM DTT buffer solution, pH 7.4. Kinetics of aggregation was monitored using Thioflavin T (ThT) fluorescence assay. Morphology of aggregates was visualised by atomic force microscopy and secondary structure was tested by infrared spectroscopy.

Results: The presence of methylene blue leads to reduced ThT fluorescence intensity and results in different morphology as well as secondary structure of aggregated protein. Seeded aggregation kinetics suggests that methylene blue possibly modulates surface

properties of pre-added fibrils without affecting stability of protein monomer, but further investigation is needed to reveal the exact methylene blue mode of action.

Conclusion: Methylene blue influences SOD1 aggregation kinetics, aggregate structure and morphology.

Vilmantas Pupkis

"Cs^+ blocks high-conductance ion channels in the tonoplast of Nitellopsis obtusa: a patch clamp approach"

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Plant ion channels are crucial for various physiological functions such as sustaining electrochemical potential driven signaling pathways. Therefore investigation of plant ion channels is beneficial for obtaining fundamental knowledge and insights into cellular processes such as the generation of action potentials. For decades Characean macroalgae have proven to be a convenient and irreplaceable model system for electrophysiological investigations.

The aim of our research was to test the effect of a well-known K^+ channel blocker Cs^+ on scarcely characterized high-conductance ion channels recorded in the tonoplast of a freshwater algae *Nitellopsis obtusa.*
Plant cell wall impedes access to the plasma membrane but this issue in Characeaen algae may be overcome by using cytoplasmic droplet

technique. A cell of *N. obtusa* can be placed vertically in a solution approximately isotonic with the cell sap and "decapitated". The cell sap then flows out of the cell, in a bath solution spontaneously forming cytoplasmic droplets. The spherical droplets consist of cytoplasm covered with the vacuolar membrane – tonoplast. The tonoplast was accessed using patch clamp technique in tonoplast-attached configuration: a microelectrode was pushed against the membrane, forming a high resistance seal (usually larger than 5 GΩ). Thus ionic currents passing through ion channels in the sealed area could be easily investigated in high resolution.

Our conducted experiments indicated that Cs^+ ions block high-conductance ion channels in the tonoplast of *N. obtusa* by decreasing amplitude of inward currents. The effect is attributed to fast ion channel flickering induced by Cs^+ ions constantly associating and dissociating from the channel pore.

The fact that K^+ channel blocker Cs^+ blocks high-conductance ion channels in the tonoplast of *N. obtusa* indicates that these previously unidentified channels are K^+ channels.

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Mantas Žiaunys

"Understanding Different Pathways of Insulin Aggregation"

MANTAS ŽIAUNYS, Tomas Šneideris, Vytautas Smirnovas

Introduction: Amyloids are self-assembled, highly ordered, closely packed peptide or protein aggregates and their formation is associated with neurodegenerative diseases, such as Alzheimer's, Parkinson's and prion diseases. Amyloidogenic protein aggregation mechanism dependence on environmental factors, such as ionic strength and pH are still poorly understood.

Aim: In this work we observed unusual insulin aggregation kinetics at pH 2.4 under two ionic strength conditions that could not be explained by any currently predominant models. Our aim was to create a model that would explain such abnormal data.

Materials and methods: Insulin amyloid-like fibrils were prepared by incubation of fresh insulin solution in 100 mM phosphate buffer pH 2.4 (with and without 0.1 M NaCl) at 60 °C for 24 hours in quiescent conditions. 5.0 mg/ml insulin solutions were used in dynamic light scattering analysis and formed fibrils were examined with atomic force microscopy and infrared spectroscopy. Aggregation kinetics of insulin solutions (0.5 – 5.0 mg/ml) were recorded at constant 60°C temperature by measuring thioflavin -T fluorescence intensity. Kinetic data was analysed using "classic" and "saturated elongation" models as well as our proposed "classic" + tetramer and "classic" + "capping" model.

Results: Our proposed additional tetramer formation and aggregation center "capping" steps greatly improved the "classic"

aggregation model's ability to fit the abnormal kinetic data. The addition of salt also shifted the monomer-tetramer equilibrium towards higher oligomeric form creation.

Conclusion: At pH 2.4 under different ionic strength conditions insulin can exist in a tetrameric form, which is capable of inhibiting the elongation of fibrils by binding to aggregation centers.

Milda Boguševičiūtė

"Expression and Biochemical Characterization of Recombinant Porcine trypsin in *Pichia Pastoris*"

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Introduction: Trypsin (EC 3.4.21.4) is a pancreatic serine protease that cleaves at the carboxyl-end of lysine and arginine residues with very high selectivity. Trypsin is a medium-sized globular protein and is synthesized as inactive zymogen precursor (trypsinogen). Trypsinogen is activated by enteropeptidase, also trypsinogen possesses proteolytic activity to activate itself.

Trypsin has a wide range of applications including amino acid analysis and protein sequencing, mapping and structural studies. It has properties that make it uniquely well-suited to mass spectrometry identification, which is why trypsin is the most commonly used protease. Trypsin is able to cleave proteins into the fragments that are ideal for mass spectrometry, between 700 to 1500 Daltons, and it has a high proteolytic activity and exceptional cleavage specificity.

Aims:
1. Evaluate and determine the biochemical properties of homogenous recombinant Trypsin enzyme;
2. Evaluate recombinant trypsin application possibilities for mass spectrometry sample preparation.

Materials and methods: Recombinant trypsin was expressed as trypsinogen in methylotrophic yeast. Biochemical properties of the native porcine trypsin and recombinant analogue was compared using the mass spectrometry methodology.

Results: We have produced active and stable recombinant trypsin which proved to be a very biochemically similar to native analogue – obtained from pancreatic raw material.

Conclusions: Mass spectrometry results confirmed that native and recombinant porcine trypsin is equally effective for proteomics research application.

For Research Use Only. Not for use in diagnostic procedures.

Živilė Buivydaitė

"Electroanalytical Methods for Evaluation of Electric Field Effects on Yeast Cells"

ŽIVILĖ BUIVYDAITĖ, Povilas Šimonis, Raimonda Celiešiūtė, Rasa Garjonytė, Arūnas Stirkė

For several decades, the budding yeasts (*Saccharomyces cerevisiae*) have been used in studies of basic phenomena of eukaryotic life and some of their fundamental properties. Yeasts were used not

only for production of transgenic proteins, or fermentation in foods and beverages, but also used as microbial fuels cells for generating green electricity. Yeast cells could also be used for whole-cell bioprocesses such as biocatalysis and recombinant protein fermentation, but natural barrier functions of the cell wall and cell membrane (envelope) often retards entry of substrates and release of products. Besides barrier function, envelope provides protection from osmotic stress and is important for yeasts defense against toxic compounds, self-recognition as well as flocculation.

One of the possible techniques which could be used to improve permeability for target molecules is pulsed electric field (PEF), yet there is still a lack of sufficient data related to the effects of PEF on yeast cells. Here we employed yeast itself as an amperometric whole cell yeast sensor for the investigation of pulsed electric fields effects on yeast cells and detection of lactic acid. For the analysis, PEF-treated cells were immobilized on carbon paste electrodes which were then immersed into solution with potassium ferricyanide acting as mediator and producing measurable currents through oxidizing electrode surface. The scheme of mediated electrocatalytic oxidation of lactic acid can be represented as follows:

L-lactic acid + flavocytochrome $b2_{(ox)}$ -> pyruvic acid + flavocytochrome $b2_{(red)}$

flavocytochrome $b2_{(red)}$ + $mediator_{(ox)}$ -> flavocytochrome $b2_{(ox)}$ + $mediator_{(red)}$

$mediator_{(red)}$ -> $mediator_{(ox)}$ + e^-

Since mediator have positive charge, membranes of viable yeast cells are only weakly permeable to them, thus, only small currents were detected after addition of lactic acid into solution. We varied electric field strength (E) and pulse number (pn) as well as pulse

length (τ). After exposure to weak electric fields (E= 2 kV/cm, pn= 1) currents slightly increased up to 20 ± 0,1 nA. Further raise in electric field (E= 4 kV/cm, pn= 1) and pulse number (E= 4 kV/cm, pn= 40) resulted in even stronger currents of 28 ± 8 nA and 82 ± 19 nA respectively. When we used phenazine methosulfate instead of ferricyanide, with same pretreatment parameters (E= 4 kV/cm, pn= 40) response time was longer and smaller currents were generated (I= 30 ± 14 nA) suggesting about different membrane permeability properties.

PEF effects on yeast cell wall were also evaluated by employing ion selective microelectrode sensitive to concentration of tetraphenylphosphonium ions (TPP^+). Study of high power nanosecond duration electrical pulses effects on yeast cells, had demonstrated the following features: (i) The study of TPP^+ ions absorption rate by yeast cells is an effective method for detection of short duration electric pulse influence on yeast cell wall properties; (ii) Shortening of the electric pulse duration makes it possible to achieve more homogeneous electrical treatment of yeast cell clusters and by this way to increase the effectiveness of single cell permeabilization; (iii) The significant acceleration of TPP^+ ions absorption rate (up to 65 times) can be achieved without any influence on the vitality of the cells.

We conclude that electroanalytical tools can be effectively used as a useful tool for investigation of various cellular responses after PEF treatment. After exposure to PEF, permeability of membranes as well as of cell walls was improved thus suggesting PEF as a tool for biocatalysis enhancement.

Tomas Šneideris

"Effect of Temperature and Denaturant Concentration on Elongation of Distinct Mouse Prion Protein Fibril Strains"

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Introduction: Prion diseases is a group of fatal neurodegenerative disorders that affect many mammalian species. These diseases have three distinct causes: in the most of cases (85%) occurrence of these diseases in humans is spontaneous, less likely (10%) it is a result of mutations in the PrP gene *PRNP* and in a rare cases (5%) it is an infection due to exposure to prions via medical procedures, prion-contaminated food or cannibalism. One of the most interesting aspects of prion diseases is existence of multiple "strains", which lead to different disease phenotypes that are distinguished by distinct clinical signs, incubation time and neuropathology. However, it is still not clear what factors lead to polymorphism of prions or how many polymorphs can be formed. The main causative agent of prion diseases is thought to be the aggregated form of native prion protein ($PrP^{C)}$, known as PrP^{Sc}. The infectivity of prion diseases is based on PrP^{Sc} ability to self-propagate by binding to PrP^{C} and inducing its conformational conversion to the PrP^{Sc} state. One of the possible mechanisms by which PrP^{Sc} self-replicates is the elongation of amyloid fibrils.

Aim: The aim of this study is to determine and compare the effect of temperature and denaturant concentration on elongation kinetics of distinct mouse prion fibril strains. Results of this research could contribute to the deeper understanding of amyloid polymorphism and self-propagation.

Results and Conclusions: Distinct strains of mouse prion protein fibrils display different profiles of elongation kinetics under various environmental conditions. In some cases, unusual profile of elongation kinetics point to possible remodeling of existent amyloid fibrils or presence of multiple polymorphs in a sample, however, due to complexity of the aggregation process it is difficult to determine the real cause and deeper investigation is needed.

Romuald Stanilko

"Impact of Environmental Factors on Insulin Aggregation Kinetics"

ROMUALD STANILKO, Tomas Šneideris, Vytautas Smirnovas

Introduction: Amyloid fibrillation is directly implicated in several degenerative pathologies such as Alzheimer and Parkinson diseases. Recent studies have demonstrated that the epigallocatechin-3-gallate (EGCG), the main and most significant polyphenol in green tea, potently inhibits the formation of amyloid fibrils. However, epidemiological inferences are sometimes conflicting and *in vitro* and *in vivo* studies may seem discrepant. In this study, we have investigated the influence of EGCG at different environmental conditions on insulin fibril formation. Fibrillation of insulin are meaningful not only for the quality control of pharmaceutical insulin batches but also to gain a deeper understanding of the amyloid fibrillation process.

Aim: to study insulin fibril formation at different environmental conditions and determine the EGCG effect on insulin fibrillation.

Materials and methods: Different recombinant human insulin concentrations were incubated in 20 % acetic acid buffer

(pH=1.85, 100 mM NaCl) and 100 mM phosphate buffer (pH=2.4, 100 mM NaCl). In this work the effect of 2 non-identical forms of EGCG (oxidized and reduced) were observed. Oxidized form was prepared by dissolving EGCG in 10 mM phosphate buffer (pH=7.0) and incubating for 24 hours at 37°C, while reduced form stock was freshly prepared in 10 mM phosphatic buffer (pH=7.0). Insulin aggregations were measured using a Thioflavin T (ThT) binding assay.

Results: Concentration dependent amyloid-like fibril formation profile shows significant difference between acetic acid and phosphate buffers. What is more, in phosphate buffer the oxidized form of EGCG shows noticeably stronger inhibition behavior to the fibril formation than that of the freshly prepared EGCG.

Conclusions: Amyloid-like fibril formation and efficiency of inhibitors strongly depends on environmental conditions. The detailed molecular mechanism is still unclear and requires further investigation.

Kamilė Šepetytė

"Analysis of efflux activity in *Listeria monocytogenes* cells"

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Introduction: *Listeria monocytogenes* is an opportunistic foodborne Gram-positive pathogen causing serious human infections. These bacteria are widely distributed in the environment and are the third most common cause of death from food poisoning. Because of

efflux pumps *L. monocytogenes* is a multidrug resistant pathogen, not sensitive to many antimicrobial compounds, including tetracycline, amoxicillin, benzalkonium chloride. Efflux pumps are membrane transporters which can extrude out of the cells various toxic compounds. Knowledge about the regulation of activity of these pumps could enhance the efficiency of treatment. One of ideas how to control the efflux is to usethe pump inhibitors.

Aim: To assessthe influence of potential inhibitors on the interaction of *L. monocytogenes* cells with efflux indicator Tetraphenylphosphonium (TPP^+) ions.

Materials and methods: TPP^+ is universal efflux pump substrate in prokaryotic and eukaryotic cells. Electrochemical analysis was used to follow the distribution of TPP^+ ions between the cells and the incubation medium. We used well-known inhibitors of Gram-positive cell transporters, such as Chlorpromazine, Reserpine and Verapamil, to analyze the efflux activity in *L. monocytogenes*. In addition, we explored effects of Phe-Arg-β-naphthylamide (PAβN), which is known as an inhibitor of RND family pumps in Gram-negative bacteria.

Results: Accumulation of TPP^+ ions was analyzed at different incubation conditions. The cells, incubated in 100 mM phosphate medium, were sensitive to all mentioned inhibitors and temporally accumulated the increased amounts of TPP^+. In arsenate-containing medium the period of temporal additional accumulation of TPP^+ was registered. This stage of interaction between *L. monocytogenes* and TPP^+ was not observed in phosphate medium.

Conclusions: All used Efflux pump inhibitors increased the accumulation of TPP+ ions in *L. monocytogenes* cells. PAβN demonstrated the efflux inhibiting effect not only on Gram-negative, but also on *L. monocytogenes* cells.

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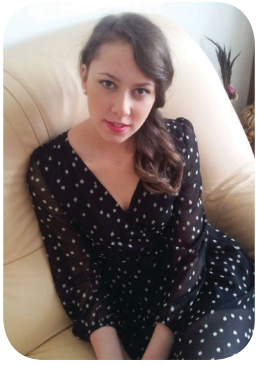
Elžbieta Kulicka

"Polymorphism of Mouse Prion Protein"



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Introduction: Prion diseases, also known as the transmissible spongiform encephalopathies (TSEs), are a group of fatal neurodegenerative disorders that affect humans and animals. These diseases are associated with conformational conversion of the cellular prion protein PrP^{C}, into an oligomeric β-sheet rich form PrP^{Sc}. One of the most intriguing features of prions is their ability to form different strains, leading to distinct phenotypes of TSE diseases. However it is still not clear what factors lead to polymorphism of prions or how many polymorphs can be formed.

Aim: The aim of this research is to study aggregation of mouse prion protein under various environmental conditions that could possibly induce formation of distinct amyloid aggregates (strains) *in vitro.*

Materials and methods: The mouse prion protein (MoPrP 89-230) was expressed in *E. coli* and purified by Ni^{+} affinity chromatography. To prepare fibrils, monomeric prion protein was diluted to concentration of 0,5 mg/mL in 50 mM phosphate buffer (pH 6)

containing 2 M GuHCl and incubated under variuos environmental conditions. The morphology and secondary structure of formed fibrils were determined by atomic force microscopy and infrared spectroscopy respectively. The resistence to denaturant (GuSCN) were observed by platereader, measuring the Thioflavin T fluorescence. Fractional loss of signal at increasing denaturant concentrations corresponds to the fraction of MoPrP dissociated from amyloid fibrils.

Results: Currently four distinct strains were propagated that displayed different structural, morphological aspects and resistance to chemical denaturation.

Conclusions: Different temperature and agitation conditions lead to formations of distinct strains of mouse prion protein. Results of this research could contribute to the deeper understanding of amyloid polymorphism.

Vaida Paketurytė

"Thermodynamic Analysis of Benzenesulfonamides Bearing Oxopyrrolidine Moiety Binding to Human Carbonic Anhydrases"

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Introduction: Studies of the interaction between proteins and small molecules are important due to two main reasons: first, this

is helpful for rational drug design and, second, it is necessary to deepen the understanding about molecular interactions. In this study, target proteins are human catalytically active carbonic anhydrase (CA) isoforms. It is well know that sulfonamides bind to the zinc ion in the active site of all CAs, but different substitutions of benzenesulfonamides (or other suflonamides) have different affinity. Tracking the changes of the chemical structures of compounds and affinity for CAs could be useful for the lead compound (potential drug) optimization.

Aim: To determine the affinity and selectivity of new synthesized oxopyrrolidine-containing benzenesulfonamides with human catalytically active carbonic anhydrases.

Materials and methods: The binding constants were determined by the fluorescent thermal shif assay (FTSA) and isothermal titration calorimetry (ITC) methods.

Results: In this study, 2,6-dimethylbenzenesulfonamides with/without halogens in *ortho* position and with different pyrrolidin-2-ones in *para* position were synthesized and observed affinities to 12 catalytically active recombinant human CA isoforms were determined by the FTSA. Moreover, ITC method was also used to know if the binding is driven by enthalpy or entropy.

Conclusions: The correlation between chemical structures of compounds and affinities to CAs was determined and will be presented in a poster.

Andrius Sakalauskas, Justina Jurgelevičiūtė

"Characterisation of Sup35NMp Amyloid-like Fibrils and Their Infectivity *in vivo*"

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Introduction: Prion particles are associated with infectious prion diseases in mammals and inherited phenotypes in *Saccharomyces cerevisiae*. Sup35p, found in yeast, undergoes prion-like fibril formation and infects *S. cerevisiae* cells by inducing *[PSI+]* phenotype. To understand why aggregates differ in infectivity, their physical properties are currently being intensively studied.

Aim: The aim of this research was to analyze the characteristics and formation of Sup35NMp fibrils *in vitro* and their infectivity in *S. cerevisiae* cell for better understanding of prion diseases in mammals.

Materials and methods: Recombinant Sup35NMp was purified using Ni^{2+} affinity chromatography followed by a SP-Sepharose IEX chromatography. Aggregation studies were carried out in several pH 7.4 buffer solutions. Fibril formation kinetics were monitored by measuring intensity fluorescence of ThT using Varian Cary Eclipse fluorescence spectrophotometer. Samples were imaged using a Dimension Icon Bruker AFM. The stability of fibrils were monitored

using different GuHCl concentrations in Synergy H_4 Hybrid Multi-Mode microplate reader. Sup35NMp fibrils were transformed into *S. cerevisiae* using electroporation.

Results: Experimental analysis showed that Sup35NMp fibrils formation kinetics depend on temperature. The stability of formed fibrils were examined revealing differences of strains. Moreover, we have found that the synthetic Sup35NMp fibrils induce the *[PSI+]* prion phenotype in *S. cerevisiae* cells, while the induction efficiency depends on the length of the particles.

Conclusion: This research revealed that the Sup35NMp fibril strains formed in vitro can be characterized by different aggregation rate and stability. Moreover, the infectivity of *[PSI+]* phenotype *in vivo* depends on the Sup35NMp fibril characteristics. A better understanding of the prion fibrils formation characteristics and their infectivity mechanisms will lead to effective cure of mammalian and human prion diseases.

Ignas Sabeckis, Deimantė Noreikaitė

"Beta Glucan Extraction from *Gyromitra* spp. Mushroom"

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Mushrooms are known to contain vast amounts of glucans. Our chosen research object *Gyromitra* spp. mushroom was selected for the following reasons: wide availability, esthetical approach and its uninvestigated properties. Beta glucan has unique rheological properties and can be used as food additive or raw material for edible film production. Also, glucans are notable for having antioxidant, antitumor, immunostimulant, antimicrobial, cardioprotective and hepatoprotective properties. Three different methods of extraction were used to quantify beta glucans extracted from *Gyromitra* spp. mushroom. First method was aqueous extraction. In this method we used milled mushroom powder and water solution in ratio 1:9, heated at 100°C temperature for 4 hours. While using alkaline extraction the ratio of milled mushroom powder and NaOH 1 % solution was 1:15 and required no additional heating. Third method, sonication assisted extraction, was carried out using water and milled mushroom solution while using homogenizator at 80 % amplitude (66,626 kJ). After each method samples were centrifugated and supernatant was separated from hard phase particles. 50 ml of each recovered solution was precipitated using three times ethanol and the

precipitant was recovered using centrifugation. Lastly, drying phase was carried out using 50 °C temperature for two days. The results showed, that best method for beta glucan extraction was sonication. It demonstrated, that the biggest yield was 0,8289 g compared to other methods, 0,4796 g alkaline extraction, 0,9348 g aqueous extraction considering the ratio. Furthermore, sonication assisted method can be used for extracting higher amounts of beta glucans while using less raw material.

Robertas Stankevič

"Comparing Dynamical Time Warping and Cross Recurrence Plot Approaches in Geological-Paleontological Time Series Synchronization"

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Introduction: Geological correlation is the most fundamental geological research problem. It is devoted to the synchronization of a large number of spatially separated records, in order to construct a comprehensive record of the geological developments of a planet. The new development in this field is cross recurrence plots and dynamical time warping analyses, which could be used in deciphering nonlinear distortions in the spatially distributed coherent dynamics.

Aim of our research is to compare different algorithms for synchronizing pairs of geological time series.

Materials and methods: Here we compare pairs of time series from real geological data gained from core drills and simulated data

using deterministic and random mathematical functions. Each algorithm tries to find near-diagonal synchronization line which should correspond to the real line the best. First two algorithms are greedy. First searches nearest points of similarity on each iteration. Second algorithm uses moving window and choose median point of all similarity points which fall into the window. Third algorithm is called dynamic time warping (DTW) and it calculates minimal costs of the ways from the corner of the recurrence plot to all other points, including diagonally opposite corner point.

Results: Best results to synchronize simulated data are achieved with DTW algorithm. First algorithm fails to move vertically or horizontally in the areas where there are many similarity points, this results in finding synchronization line which is far from real. Second (median) algorithm results in more accurate synchronization line, although it depends on parameters used. DTW algorithm recognize synchroline at the best, however it fails to choose accurate path inside a laminar zone (as in the case of comparison of Vilkaviškis-134 and Ledai-179 cores).

Conclusion: Dynamic time warping algorithm is shown to be sufficiently accurate for synchronizing geological time series, and can be further improved for better synchronizing of rectangular high-similarity zones in cross recurrence plots.

Lukas Gedvilas

"Pre-Sowing Seed Treatment by Cold Plasma and Electromagnetic Field Stimulates on Trichome Formation on Picea abies Needles"

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Norway spruce is subject to increasing concerns about forest decline. That underlies the need to develop novel technologies that could lead to improved seedling performance and resistance. Seed treatment by electromagnetic field (EMF) or cold plasma (CP) is recognized as an innovative tool for enhancement of seed germination and early seedling growth. Long time observations have revealed that Norway spruce (*Picea abies* (L.) Karst.) seed treatments with CP and EMF induce changes in seed germination, stimulate seedling growth and branching, and increase the length of needles. The aim of this study was to estimate the effects of pre-sowing seed treatment with CP and EMF on trichome formation on Norway spruce needles.

The obtained results indicate a significant increase of average number of trichomes on a young (current year) needles (by 2.5 times) and older previous year needles (by 1.93 times) developed on seedlings grown from seeds treated by CP for 2 minutes (CP2). Seed treatment by EMF (5 and 10 min) induced later positive effect - the trichome number on young needles was increased by 1,97 and 3,35 times, respectively. However, no significant effects on older

needle trichome formation were detected by the used treatments, except CP2. Moreover, CP (5 min) and vacuum had negative effect on trichome formation on young needles - the average number of trichomes decreased by 2.8 and 5 times, respectively. Electron scanning microscopy did not detect any differences in trichome surface structure of treated and control seedling needles.

Our data show that seed treatment by CP and EMF may be a promising tool of increasing trichome formation on *Picea abies* needles. Trichomes are protective structures involved in synthesis and storage of volatile oils and other secondary metabolites. The reported effect may improve plant communication, strengthen stress response and resistance to pests and pathogens.

Reda Eglinskaitė

"Toxicity assessment of Gineitiškės lake inflow and outflow using luminescent bacteria *Aliivibrio fischeri*"

REDA EGLINSKAITĖ, Virginija Kalcienė, Vaidotas Valskys

Bioassays based on reduction in bioluminescence of marine bacterium *Aliivibrio fischeri* have become widely used in sediment toxicity testing because the luminescent system is highly sensitive to certain contaminants even at micro quantities.

The aim of this research is to assess the toxicity of surface water and sediment from Gineitiškės lake inflow and outflow using biotest based on luminous bacteria *A. fischeri*. The toxicity of surface water and different sediment fractions were evaluated after 1, 5, 15, 30 minutes of exposure.

The results showed that *A. fischeri* bacteria light output intensity was inhibited significantly up to 44 % ($p<0.05$) by surface water samples from Gineitiškės lake inflow and outflow after 1-15 min. Significant differences in toxicity between inflow and outflow water samples were not determined. The sediment pore water of the lake inflow inhibited bacterial luminescence significantly ($p<0.05$) only after the first minute of exposure and stimulated it after 15 and 30 minutes. Meanwhile, sediment pore water of the lake outflow inhibited luminescence during all exposition time and inhibitory effects were enhanced with increasing exposure duration. This suggests that the outflow sediments are contaminated with water-soluble toxic substances. The elutriates of sediment samples from Gineitiškės lake inflow and outflow did not showed a statistically significant toxic effect ($p<0.05$). Meanwhile acetone extracts of sediments inhibited *A. fischeri* luminescence significantly ($p<0.05$). This indicates that the inflow and outflow sediments are contaminated with hydrophobic toxic substances. The treatment of acetone extracts with copper produced a statistically significant ($p<0.05$) reduction in the toxicity to *A. fischeri* bacteria. This suggests that the toxicity of sediment acetone extracts is partly due to the presence of sulfur in the sediment.

In conclusion, analysis of different fractions of sediments toxicity revealed that sediments from both lake sites (inflow and outflow) are contaminated with hydrophobic toxic substances. Chemical analysis is needed for the detection and confirmation of sediment contamination.

Laura Songailaitė

"Molecular Characterization of *Bartonella* Bacteria in Deer Keds (*Lipoptena*) from Lithuania"

LAURA SONGAILAITĖ, Kamilė Klepeckienė, Algimantas Paulauskas, Jana Radzijevskaja
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Introduction: *Bartonella* bacteria can live inside cells and in isolated body areas protected from the immune system of host and antibiotics. *Bartonella* spp. caused disease in humans and animals named bartonellosis. Deer keds are transmitted *Bartonella* infection to the deer and humans by directly biting. In ruminants are commonly found *Bartonella schoenbuchensis*, *Bartonella bovis*, *Bartonella capreoli* and *Bartonella chomelii* species.

Aim: We aimed to detect and molecularly characterize *Bartonella* spp. in two species of deer keds *Lipoptena cervi* and *Lipoptena fortisetosa* using PCR assay and sequence analysis based on *gltA* gene and 16S-23S rRNA intergenic spacer region (ITS).

Materials and methods: In total 292 deer keds were collected from 5 roe deer, 1 red deer and 2 moose in Lithuania. DNA from deer keds was isolated using Genomic DNA Purification Kit. A partial region of *gltA* gene and ITS region of *Bartonella* were amplified using nested PCR. PCR products of ITS region were sequenced and subjected to BLASTn identity searches in the GenBank database. The phylogenetic analysis was constructed using MEGA 6 software.

Results: *Bartonella* DNA was detected in 48 (98%) from 49 pools and 13 (74%) from 50 pools of *Lipoptena cervi* and *Lipoptena fortisetosa*, respectively. *Bartonella* positive deer keds were obtained from

all collected animals. Seven different sequence variants were identified based on ITS region 800 bp sequence analysis. Obtained *Bartonella* sequences showed 96-99 % sequence similarity with *B.schoenbuchensis* and *B.chomelii* species.

Conclusion: The present findings show high prevalence of *Bartonella* pathogens in deer keds *Lipoptena cervi* and *Lipoptena fortisetosa* in Lithuania. Sequence analysis reveal the presence of *Bartonella* strains closely related to *B.schoenbuchensis*, which commonly causes alopecia and dermatitis in ruminants and deer ked dermatitis in humans.

Agnė Zdaniauskienė

"Shell-Isolated Nanoparticle-Enhanced Raman Spectroscopy as a Means to Investigate Yeasts"

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Surface enhanced Raman scattering (SERS) technique is a powerful tool for *in situ* studies of surface and interface of proteins with ultrahigh sensitivity and excellent spectral resolution. Large enhancement can be provided by roughened surfaces and nanoparticles of Ag, Au or Cu metals. Recently, Ag and Au nanoparticles have been used for identification of yeasts by SERS approach; however, high luminescent background and direct interaction of cells with metal colloids were the main disadvantages. Shell-isolated nanoparticles were found to eliminate these problems. Inert shells surround Au

or Ag cores, protecting them from degradation, aggregation and increasing stability. The shells also prevent chemical and electrical contacts among plasmonic cores and probe substrates, analytes or environment. Measurement technique with such particles spread over a surface of analyzed material is named Shell-Isolated Nanoparticle-Enhanced Raman Spectroscopy (SHINERS).

Yeasts *Metschnikowia* spp. are capable to produce a red pigment when iron (III) ions present in the growth media. They are potential biocontrol agents against various pathogenic microorganisms. SHINERS method employing synthesized spherical gold nanoparticles with 40 ± 5 nm core size and SiO_2 shell of 5 nm thickness allowed to obtain significantly enhanced SHINERS spectra of *Metschnikowia* spp. compared to the Raman spectra.

Jokūbas Krutkevičius
"Biodegradation of Polyether Polyurethane"

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Polyurethane is widely used in consumer products due to its excellent physical properties and resilience to aging and degeneration. On the other hand, these features make waste containing polyurethane a troublesome problem from the environmental point of view. Some types of polyurethanes such as polyester polyurethane are amenable to microbiological degradation. However, microbial degradation of polyether polyurethane is slow and rarely observed due to its unique structure. Currently, only few species of bacteria

and fungi have been described in the literature to be able to degrade the latter functional group in polyurethane.

The aim of this research is to isolate soil origin microorganism that are able to degrade polyether polyurethane and to investigate the enzymes responsible for this specific degradation. Microorganisms from Lithuanian soil were isolated by screening their ability to grow on minimum cultivation medium enriched with polyurethane as carbon and nitrogen sources. Ten strains of bacteria were isolated as potential polyurethane degraders and two of them were identified as *Bacillus cereus* and *Achromobacter denitrifinacs* using phylogenetic analysis of sequenced 16S ribosome gene DNA. Further study is focused on identifying specific enzymes produced by these bacteria which are responsible for the degradation of ether functional group. SDS-PAGE and evaluation of physical degradation of polyurethane were used for evaluation. The obtained results in more detail will be presented during the poster session.

Dominykas Murza

"Generation of Zika Virus-Like Particles in *Saccharomyces Cerevisiae*"

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Introduction: The insights about virulence of Zika virus has initiated the demand for novel methods to fight the spread of infections. Current research has shown that recombinant technology holds great potential to provide mankind with effective vaccines against Zika virus.

Aim: We have set out to assess the capability of genetically engineered *Saccharomyces cerevisiae* to biosynthesize Zika virus structural proteins that self-assemble into virus-like particles (VLPs). VLP-based vaccinations do not introduce viral genetic material into human organism and therefore reduce the risk of complications in comparison with attenuated vaccines. Furthermore, VLPs are resistant to freeze-drying which allows for easier transportation to distant countries. Availability of vaccination might be further enhanced due to cheap cultivation of recombinant yeast strains which are also more versatile in glycoprotein expression than bacterial expression systems.

Materials and methods: In this project we used *Saccharomyces cerevisiae* strains Fh4c, 214, 214Δpep and Gcn2 as hosts, pFx7 and pFGG3 as expression vectors. The expression of a variety of full and shortened Zika virus structural proteins was followed by ultracentrifugation using gradients of sucrose and CsCl. The protein fractions with least impurities were observed by means of electron microscopy.

Results: We were able to show the generation of Zika VLPs in yeast strain 214 by coexpressing E and prM genes in pFGG3.

Conclusion: These initial results indicate the need of further expression and purification optimization. The obtained pure proteins would enable us to proceed to their immunological characterization.

Gabija Aleknavičiūtė

"Investigation of Bacterial Laccase from *Bacillus pumilus*"

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Majority of chemical processes used in the industry have been harmful to the environment; therefore, more attention is being focused on the development of eco-friendly products. An alternative for the chemical process can be a biocatalytic process, whereas enzymes are used as catalysts for the chemical reactions. This environmental-friendly process produces the desired products without the formation of side materials and large amounts of waste products. One of such biocatalysts are laccases, which are very attractive enzymes in the industry (textile, pharmaceutical, waste disinfection, bioremediation, *etc*.).The aim of this research is to express bacterial laccase in the yeast *Saccaromyces cerevisiae*. The yeast was chosen due to their easy and inexpensive cultivation, production of targeted proteins in higher yields and their eco-friendliness. For protein extracellular secretion alfa factor and inducible promoter were used. For selective maintenance of recombinant clones the resistance system to formaldehyde was implicated. Currently, the cultivation parameters are under investigation. In our experiments, various genetic engineering methods were used: for the fusion of DNA fragments by the homologous recombination method, transformation of plasmid DNA into *E.coli* and *S. cerevisiae* colony PCR, plasmid DNA extraction, laccase activity assay, *etc*. The obtained in more detail will be presented during the poster session.

Eglė Narmontaitė

"Investigation of Pyridine Degradation in *Rhodococcus rhodochrous* PY11"

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Introduction: Pyridine and its derivatives occur in nature as components of biological systems. They are widely used in chemical pharmaceutical and oil industries and are major environmental pollutants. Due to their heterocyclic structure, these compounds easily reach the biosphere in industrial waste waters and cause the danger for living organisms. Although research has shown that a number of organisms are capable of transforming heterocyclic aromatic compounds, the mechanism of the cleavage of the pyridine ring still remains unclear. *Rhodococcus* strains are capable of degrading various xenobiotic compounds including pyridine due to their wide range of oxidative enzymes. *Rhodococci* typically harbour large linear or circular plasmids which contain a large number of catabolic genes.

Aim: It was previously discovered that *Rhodococcus rhodochrous* PY11 is capable of using pyridine as a sole source of carbon and nitrogen. The aim of this work was to investigate the degradation of pyridine in *R. rhodochrous* PY11 strain and to identify the proteins and genes involved in this process.

Materials and methods: For bioconversion experiments **R. rhodochrous** PY11 cells were grown with pyridine or succinate (control) in mineral medium at 30°C for 19 hours. The progress of conversion was monitored by changes in UV absorption spectrum in 200-320 nm range. The sequences of peptides of pyridine

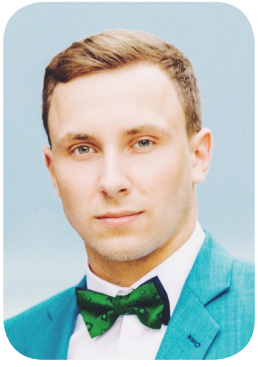
induced proteins were determined at Proteomics centre in Vilnius University. Phylogenetic analysis of identified 40kDa and 41kDa proteins was performed using Mega 6.0 program (Saitou and Nei, 1987).

Results: The bioconversion results using *R. rhodochrous* PY11 cells indicated that the degradation of pyridine is an inducible process. Two of induced proteins were determined to be 40 kDa and 41 kDa in size. They are encoded by *orf22* and *orf80* that are located in pPYR catabolic plasmid. The phylogenetic analysis of 40 kDa and 41 kDa proteins revealed that they are mostly related to amidohidrolase and luciferase, respectively.

Conclusion: The identified proteins of *Rhodococcus rhodochrous* PY11 will help to determine the pyridine degradation pathway.

Žymantas Venckus

"Bacterial Lipoxygenase Expression in *Saccharomyces cerevisiae*"



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The production of enzymes has been the main pursuit of modern biotechnology for the past few decades. The demand for traditional industrial enzymes continues to grow due to their ecological and economical advantages. Consequently, there is an increasing need of the new biological catalysts that can introduce novel application possibilities. One of such, is the biocatalytic synthesis of polyols from vegetable oils. Among the enzymes which can catalize this

type of reaction, lipoxygenases are of great interest due to their unique features. Although lipoxygenases exist in almost every living organism, they differentiate by the type of substrate they utilize. This research specifically focuses on the biosynthesis of bacterial oleate 10S-lipoxygenase from *Pseudomonas aeruginosa* in heterologous yeast systems. *Saccharomyces cerevisiae* was chosen for this experiment as cell factories due to their embodied specific properties: fast and cheap cultivation, high titer and recognition as environmentally friendly organism.

The purpose of this study was to develope an expression system for extracellular lipoxygenase secretion in *Saccharomyces cerevisiae*. To achieve that, a DNA construct containing target gene was created together with endopolygalacturonase (EPG) signaling peptide specific to *Kluveromyces marxianus.* Additionally, his-tag approach was implicated in pursuance for protein purification. Experimental investigation consisted of various genetic engineering, protein analysis and microbiological methods. The more detailed results will be shown and discussed in the poster presentation.

Simas Jasiūnas

"Identification of Colistin Resistance Gene Origin in Escherichia Coli Isolated from Migratory Bird"

SIMAS JASIŪNAS, Julija Armalytė, Modestas Ružauskas, Edita Sužiedėlienė

Introduction: As antimicrobial resistance is becoming a major threat to global health, colistin remains as one of the very few antibiotics that can still be effectively used against multi-drug resistant bacteria. However, an outbreak of plasmid-mediated colistin resistance, conferred by *mcr-1* gene, was recently reported.

Since the *mcr-1* plasmids can be transferred between different species of bacteria, it causes concern of the emergence of bacteria resistant to all known antibiotics. Therefore, to identify and understand the mechanisms of *mcr-1* spread is crucial for reducing fatalities caused by untreatable bacterial infections.

Aim: To identify the origin of *mcr-1* from *E. coli* isolated from faeces of *Larus argentatus* in Kaunas city dump. To the best of our knowledge, it is the first known occurrence of *mcr-1* in Lithuania.

Materials and Methods: To ensure that the *mcr-1* is located in a plasmid, it was transferred to another strain via conjugation. PCR was performed to confirm the presence of *mcr-1* and to find other antibiotic resistance genes in the plasmid. NCBI GenBank Database was screened for occurrences of *mcr-1* gene using BLAST (100% identity and coverage cut-off). Potential plasmids were evaluated by restriction analysis.

Results: The *mcr-1* was successfully transferred into *E. coli* K-12 laboratory strain, proving that it is located in a plasmid. Any other antibiotic resistance genes were not found in the plasmid. 143 complete plasmid sequences from NCBI GenBank database contained identical *mcr-1* sequence. By data acquired from restriction analysis of the extracted target plasmid, we can conclude, that it belongs to a group of 23 highly similar plasmids.

Conclusion: The *mcr-1* gene in *E. coli* isolated from migratory bird was found to be located in a plasmid widely spread across the world and shared between both animal vectors and human hospital patients.

Aliona Špakova

"Tailed Bacteriophage-inspired Nanotubes as a Phage Display: A Strategy Incorporating Yeast"

ALIONA ŠPAKOVA[1], Raminta Batiuškaitė[1], Eugenijus Šimoliūnas[2], Lidija Truncaitė[2], Rolandas Meškys[2], Rasa Petraitytė-Burneikienė[1]

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Introduction: The synthesis of viral structural proteins could result in virus-like particles (VLPs) formation. VLPs are particularly interesting as candidates for vaccine development. VLPs are known for their favorable structural characteristics, induction of strong immune responses and being safe templates for surface decoration by inserted immunogenic epitopes. While many icosahedral VLPs are synthesized in bacteria, the prokaryotic protein synthesis system has disadvantages such as lack of many post-translational modifications that are needed for eukaryotic proteins and contamination of purified VLPs with bacterial endotoxins. Nevertheless, icosahedral VLP platforms have been studied in detail, but rod-shaped VLPs have been mostly forgotten. Until now, there is no information regarding the generation of tailed-bacteriophage nanotubes in yeast cells.

The **aim** of this research is to generate nanotubes using bacteriophage tail proteins by employing yeast protein synthesis system.

Materials and methods: DNA sequence coding a tail tube protein of NBD2 phage was cloned into pFX7 vector. Protein synthesis was induced in *Saccharomyces cerevisiae* cells after transforming them

with the plasmid. The sucrose-purified proteins were analyzed by electron microscopy.

Results: Our work has focused on developing an alternative epitope-presenting rod-shaped platform which could be used for biomedical applications. To our knowledge, it is the first attempt to produce bacteriophage-originated nanotubes in yeast cells. Yeast protein synthesis system allowed efficient generation of high-number long and flexible nanotubes originated from NBD2 tailed-bacteriophage belonging to *Siphoviridae* family. Preliminary results of NBD2 nanotube particle investigation showed very high NBD2 nanotube stability at different conditions.

Conclusion: This work intends to show the suitability of yeast protein synthesis system to generate high-yields of stable, long and flexible nanotubes that originate from tailed-bacteriophage. The novel strategy presented here provide safer vaccine candidates compared to the VLPs synthesized in bacteria.

Arūnė Verbickaitė

"Synthesis of Rat Hepatitis E Virus Capsid Protein in Yeast and Application in Immunoanalysis"

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Introduction: Hepatitis E virus (HEV) is an important public health disease in many parts of the World. It is one of the most abundant cause of acute clinical hepatitis in developing countries. Although hepatitis E is rare in the industrialized regions, antibodies against the virus are found in human sera worldwide. HEV has variable

clinical presentations and ranges from asymptomatic carriers to fulminant hepatitis. Virus transmission is primarily via fecal-oral route but virus is also thought to be spread zoonotically. HEV has been identified in numerous animal species, including rats, swine, deer, chicken, fish and others which serving as the possible reservoir for human infections. Rat Hepatitis E (rat HEV) virus first time identified in 2010 in Germany. It belongs to the genus *Orthohepevirus* in the family *Hepeviridae*. Rat HEV is non-enveloped virus that contain positive sense RNA. ORF2 encodes the immunogenic virus capsid protein.

Aim: To create synthesis system of truncated recombinant rat HEV capsid proteins in yeast *Saccharomyces cerevisiae* and to employ the synthesized proteins in immunoanalysis of *Rattus norvegicus* sera.

Methods: Using gene engineering methods yeast expression vectors with gene coding rat HEV capsid protein was created. After yeast transformation and induction of target protein synthesis, it was purified by ultracentrifugation. Immunogenic feature of recombinant rat HEV capsid protein was verified by Western Blot with polyclonal antibodies against rat HEV.

Results: During this work recombinant rat HEV truncated capsid protein synthesis system in *S. cerevisiae* was created. The synthesized proteins variants comprising amino acids 112-645, 112-608 and 1-608 (full length of rat HEV capsid protein is 645 amino acids) were purified. It was found that protein comprising amino acids 112-608 forms virus-like particles. All purified proteins reacted with polyclonal antibodies against rat HEV in Western blot.

Conclusion: The rat hepatitis E virus capsid protein and its antigenic feature could be useful tools for a development of diagnostic ELISA tests for detection of the seroprevalence of HEV in rats and other wild animals.

Deimantė Galalytė

"Effect of Preincubation in Hypertonic Media on the Desiccation Tolerance of *Saccharomyces cerevisiae* Cells"

DEIMANTĖ GALALYTĖ, Neringa Kuliešienė, Rimantas Daugelavičius
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Introduction: *Saccharomyces cerevisiae* is a unicellular eukaryotic organism that often undergoes transition into the state of anhydrobiosis. During desiccation these cells lose the intracellular water, but still protect their structures from damages for successful recovery during rehydration.

Aim: This study was performed to explore effects of cell preincubation in concentrated metabolizable carbon sources solutions on the resistance of *S. cerevisiae* to dehydration-rehydration.

Materials and methods: Two *S. cerevisiae* strains were studied: #14 – semi-resistant, and #77 – very resistant to dehydration. The cells were incubated in 1 M solutions of glucose, lactose or glycerol for 3 h and desiccated at 30 oC for 21 h. Metabolic activity of the cells during rehydration was assayed by oxygen consumption test and fluorescence microscopy.

Results: This study revealed that 1 M lactose-pretreated cells of #14 strain were consuming 70 % and 1 M glycerol-pretreated - 90% of the incubation medium dissolved oxygen. 1 M lactose-pretreated cells of #77 strain were consuming 40 % of the dissolved oxygen, i.e., about 2-fold less than #14 strain, and 1 M glycerol-pretreated - 50 % of the dissolved O2. The most intriguing result of

this study is that after preincubation in the concentrated solutions of lactose or glycerol cells "switched" their phenotypes: #14 strain has become more resistant to dehydration than #77 one. However, such protection was not observed after preincubation in 1 M glucose.

Conclusion: Our results indicate that preincubation of the yeast cells in hypertonic solutions of glycerol or lactose highly increases their resistance to desiccation, but glucose does not have such cell-protecting effect. This effect can be metabolism-driven, because lactose and glycerol are non-fermentable carbon sources in *S. cerevisiae* cells.

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Gintarė Vaitiekaitytė

"Genotoxicity Assessment of Soil from Technogenic Pollution Sites by Application of *Allium* Bioassays"

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Introduction: Technogenic soil polluted with various contaminants can cause human health problems, especially in the vicinity of the residential areas. Heavy metal contamination is typical at the industrial sites, surroundings of oil product storage and thermal power plants. Soil evaluation by the physical-chemical analysis

combined with the analysis of (geno)toxicity biomarkers in model organisms provides information about ecological status of sites with intensive technogenic load.

Aim: evaluate technogenic soil for its genotoxicity in plant test-system employing cytogenetic bioassays.

Methods: Chemical analysis of soil samples and classification according to the hazard levels based on the total heavy metal pollution. Soil genotoxicity determined using *Allium* chromosome aberration and micronuclei assays after 24 and 48-hour of onion exposure to the soil, respectively, with the determination of mitotic index all along.

Results: Seven test-soils had the permissible level of total heavy metal contamination, the rest nine soils were classified as medium–extremely hazardous.
Onion root cell mitotic activity, regardless of the exposure time, did not differ between onions grown in the test-soils and control showing no cytotoxic effect of the contaminated soil. Mitotic index significantly decreased after 48-hours indicating a cumulative slowing of cell mitotic activity after recovery.
Significant induction of chromosome aberrations in root cells was observed in onions exposed to only three test-soils all of the permissible contamination showing the genotoxic potential of those soils not dependent on the inorganic contamination. Non-significant micronuclei induction in onions exposed to the test-soils shows no potential of contaminated soils for mutagenicity in plants.

Conclusions: Chromosome aberration induction did not depend on soil contamination, and was reversible as no formation of micronuclei was observed after recovery period. Heavy metals might be of inactive state as scarce or insignificant deleterious

effects were determined in plants exposed to the hazardously contaminated soils.

Funding: Lithuanian Research Council (MIP-042/2015).

Adelė Kaltenytė

"The Role of Efflux Pumps in the Antibiotic Resistance of Soil Isolates of Genera Pseudomonas, Stenotrophomonas, and Cryseobacterium Bacteria"

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Development of antibiotic resistance is common issue among soil bacteria witch are exposing to pesticides continuously at sub-lethal concentrations. Bacteria has a lot of mechanisms to increase its antibiotic resistance and one of them is efflux pumps. *Pseudomonas* spp., *Stenotrophomonas* spp. and *Crysebacterium* spp. were studied using Minimum Inhibitory Concentration Assay in order to evaluate the role of these efflux pumps in the antibiotic resistance of soil bacteria. Bacteria were extracted from soil found in ecological and intensively chemisized farms. Experiments were performed by combining antibiotics and efflux pump inhibitors. Efflux pumps played a role in antibiotic resistance in all bacterial strains from both ecological and intensively chemisized farms in bacterias from *Pseudomonas* genus and where not an essential factor for antibiotic resistance for bacteria of the genera *Stenotrophomonas* and *Crysebacterium* in either of farms. The distinction between ecological and intensively chemisized farms was not observed supposedly because continuous use of pesticides has heavily contaminated soil and ground water system.

Alesia Melnikava

"Creation of transgenic plants expressed *acdS*-gene of bacteria *Pseudomonas putida*"

A. A. MELNIKAVA, E. A. Khramtsova

Introduction: Ethylene is an essential gaseous plant hormone. It is responsible for many aspects of plant growth and development, but under stressful conditions excessive amounts of ethylene are produced which has negative impact on plant organism. The synthesis of ethylene in plants is directly proportional to the concentration of its precursor 1-aminocyclopropane-1-carboxylic acid (ACC). It can be cleaved by ACC deaminase produced by various rhizobacteria, therefore level of ethylene in stressed plants decreases. The expression of ACC-deaminase (*acdS*) gene in transgenic plants is an alternative approach to overcome the ethylene-induced stress without the necessity for exogenous application and uptaking of chemical inhibitors.

Aim: Current study was conducted with the aim to create transgenic tobacco plants which express *acdS*-gene of bacteria *Pseudomonas putida*.

Materials and methods: The *acdS*-gene was amplified by PCR and then cloned into pBI121 vector under the control of the cauliflower mosaic virus (CaMV) 35s promoter. Used primers: (Fatg) 5'-tccggatccatgaacctgaatcgttttraacgttatc-3'; (Rtga) 5'-tccggatcctcagccgttgcgraacargaag-3'. Then, the transgenic construct was integrated into the cells of *Agrobacterium tumefaciens* AGLo. Integration into the genome of *Nicotiana tobacco* was conducted using the floral-dip method for *Agrobacterium*-mediated plant transformation. Monitoring of *acdS*-gene presence in

received recombinant plants was carried out by PCR with specific primers to acdS-gene. Expression level of transgene was measured by real-time PCR. 1 µg total plant RNA was used for synthesis of 1st strand cDNA and followed by RT-PCR. Used primers for real-time PCR: (forward) 5'-atgaacctgaatcgttttgaacgttatc-3'; (reverse) 5' – cactgttgcagtcttcacgtttg-3'.

Results and conclusion: As a result, *acdS*-gene of bacteria *P. putida* has been observed to be expressed at detectable level in recombinant plants *N. tabacum*. In comparison with the reference gene the expression level of target transgene was 3,87 times more. So, it can be expected that transgenic plants with *acdS*-gene expression would be tolerant to different stress conditions.

Rūta Žemaitytė

"Synthesis of Human Parvovirus B19 Coat Protein VP2 in Yeast and Generation of Virus-Specific Polyclonal Antibodies"

RŪTA ŽEMAITYTĖ, Paulius Lukas Tamošiūnas, Aliona Špakova, Martynas Simanavičius, Indrė Kučinskaitė-Kodzė, Rasa Petraitytė-Burneikienė

Institute of Biotechnology, Life Sciences Center, Vilnius University, Vilnius, Lithuania

Introduction: Infection with human parvovirus B19 can cause a wide spectrum of symptoms, most commonly recognized as a childhood rash. Virus is not lethal for adults, while infection of developing foetus can result in death. While the capsid of B19 consist of two structural proteins, the major VP2 protein can result in virus-like particles (VLPs) in yeast without the presence of other viral proteins. Self-assembled VP2-VLPs can be used for generation of antibodies suitable for B19 virus diagnostic applications.

Aim: To synthesize viral structural protein VP2 in *S. cerevisiae* cells and generate polyclonal antibodies (pAbs) against yeast-expressed VP2 protein of human parvovirus B19.

Materials and methods: B19 VP2 protein coding DNA sequence was cloned into pFX7 plasmid. Yeast expression system was used to produce VP2 of B19 virus following protein purification under native conditions. The recombinant B19 proteins were found to self-assemble into VLPs as evidenced by electron microscopy. Finally, mice immunization with B19 VP2 resulted in generation of polyclonal antibodies (pAbs) specifically recognizing VP2 protein against which they were made, as proved by immunoblotting.

Results: VP2 protein of B19 was efficiently produced in yeast. Electron microscopy analysis proved the self-assemble of VP2 into VLPs. The diameter of icosahedral virus particles was 30 nm, which is similar in size of the native human parvovirus B19. For immunogenicity analysis and generation of VP2-specific antibodies, mice were immunized. Novel pAbs specifically recognized VP2 protein of B19 in Western blot, making them a promising tools for diagnostic applications.

Conclusions: To conclude, yeast-expressed recombinant VP2 protein of human parvovirus B19 self-assembled into VLPs. The purified VP2 were shown to be immunogenic in mice generating specifically-reacting pAbs. Further investigations will have to find out if the pAbs can be used for B19 virus detection.

Monika Stravinskaitė

"Heavy Metal Pollution Evaluation in Soil by Application of Ecotoxicological Classification and *Allium* ISSR Analysis"

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Introduction: Metals are the natural constituents of the earth's crust, and even heavy metals are naturally present in the soil. Nevertheless, in most of the industrial sites the amount of heavy metals in topsoil is increased due to human activities. Such topsoil might be harmful to life, therefore it is important to evaluate its genotoxic effect in model organisms by DNA biomarker analysis.

Aim: To classify topsoil using the ecotoxicological risk assessment method and evaluate its genotoxic potential using plant ISSR analysis.

Methods: 15 potentially harmful elements (PHEs) were determined in 7 landfill and 13 industrial soil samples by chemical analysis. Assessment of the soil hazardousness and risk classification was performed using the total contamination index Z and ecotoxicological risk index RI. ISSR profile in roots of exposed onions was compared to the unexposed onions for detection of DNA changes. Multivariate methods were used for statistical analysis of the results.

Results: 4 soils were assigned to the elevated risk categories based on RI values. Landfill soils had lower total soil contamination compared to the industrial sites that had the highest hazard levels of inorganic contamination. A significant relation was determined between hazard and risk indices. PCA for chemical data, separated the most hazardous and risky soils from the least contaminated

landfills bearing permissible levels of PHEs. PCA for molecular data, separated soils of different risk, but clustered them with the rest soils from the same site but of lesser contamination. Mantel test showed no relation between elemental and ISSR biomarker data. Cluster analysis separated the cleanest soils from the rest based on chemistry eke ISSR data.

Conclusions: All soils, bearing elevated levels of PHEs, induced DNA changes in onion roots independently on the low/high level of the soil ecotoxicological risk and hazardousness for total contamination.

Funding: Lithuanian Research Council (MIP-042/2015).

Monika Šimoliūnienė

"Characterization of Four *Pantoea* sp. Infecting Bacteriophages - New Members within the Family *Myoviridae*"

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The genus *Pantoea* comprises many versatile species of bacteria within the family of *Enterobacteriaceae*. Although some *Pantoea* isolates have bioremediation or antimicrobial potential, other bacteria within this genus have been recognized as plant pathogens causing galls, wilting, soft rot and necrosis in a variety of agriculturally relevant plants. Despite the fact that *Pantoea* species have been the subject of many studies, relatively little is

known about their predators in nature – bacteriophages, and only a limited number of reports on *Pantoea* spp. infecting phages have been published thus far.

In this study, we present a basic characterization of four novel *Pantoea* bacteriophages named AAM2, AAM37, PSKM and SSEM1. The phages were isolated from different berry shrub samples using several *Pantoea* strains. as a host for phage propagation. Phages showed a relatively narrow host range. Based on transmission electron microscopy, all aforementioned phages belong to the family *Myoviridae* and have a similar morphology: an isometric head (in diameter from about 63 to 67 nm), a neck and a contractile non-flexible tail (from about 90 to 118 nm in length) with not obviously visible tail fibers. Plating tests revealed that phages can form plaques in the temperature ranges from 7 to 37 °C, except for phage SSEM1, which could develop only at lower temperature (up to 30 °C). The phages formed plaques from 0.3 mm (phage AAM37) to 2.2 mm (phage PSKM) in diameter after 24 hours of incubation at an optimum temperature (20 °C). The bioinformatic analysis of the major capsid protein from phages AAM37 and PSKM showed 99 and 100 % identity in amino acid level to *Xanthomonas* phage vB_XveM_DIBBI, respectively. The restriction analysis of the phage genomic DNAs revealed distinct restriction profiles indicating that phages AAM37, AAM22, PSKM and SSEM1 have different genomic composition. Thus, our data confirm the existence of a group of myoviruses infecting environmental *Pantoea* strains. On the other hand, complete genome sequencing of more *Pantoea* bacteriophages (including phages mentioned in this study) and additional studies are needed to gain further insight into the morphology, physiology and genomic diversity of this particular group of bacterial viruses and their phylogenetic relatedness to other bacteriophages.

Simona Rinkevičiūtė

"The Impact of the Mulde Bioevent (Lower Silurian) on Ostracode Ecological Dynamics"

The Mulde mass extinction (~428 myr ago) was important geobiological event. Ostracodes - important component of benthic marine communities and allows understanding of the past paleobiological processes. The purpose of this work is to research the impact of the Mulde mass extinction (Lower Silurian) on ostracode ecological dynamics.

In order to achive this goal detailed sampling of the Gėluva-118 core was performed, with later processing of samples, and extraction of ostracod shells. Additionally their taxonomic identification and statistical analyses diversity in the rock samples from Gėluva-118 borehole were accomplished. During this study 58 samples (in 961,5 m - 1041 m depth interval) were taken, which span approximately two million years, - starting from the preextinction phase and the onset of the Mulde biotic event at the beggining of the Gėluva regional stage to the final recovery.

It was determined that immediately after the end of Mulde extinction in concert with a sea levels rise, the increase in abundance of individuals and diversity of taxa began and the maximum ostracode abundance was reached. Statistical analysis shows high abundance of several dominant species, which shows the decrease of complexity of ecosystems in the initial postextinction stage. However, the upper Wenlock is characterized by the decline of dominance and increase in entropy and species evenness. Probably one of the most important factors driving biodiversity and abundance change during this time interval was eustatic sea level change. It should be noted that at the higher sea levels there was higher species richness, and abundanse of their individuals.

Vilnius - Lithuania iGEM team

"SynORI – a framework for multi-plasmid systems"

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Gene copy number serves as a fundamental parameter in the dynamics of synthetic gene circuits, but is often not explicitly considered. Coupled with transcriptional and translational regulation, copy number control would offer an effective coordination and increased dynamic range of multiple gene expression. Therefore, we modified the ColE1 replicon to develop a synthetic origin of replication - SynORI - which enables the alteration of plasmid copy number. SynORI framework also incorporates a multi-plasmid regulation system based on uniquely barcoded regulatory RNA molecules, allowing to co-maintain different-group plasmids at preselected copy numbers in a standardized manner. In case certain plasmids are chosen to have a low copy number, an active partitioning system will minimize the risk of plasmid loss and increase the stability of our system. SynORI enables the creation of more complex metabolic pathways, smart assembly of protein complexes and a more precise information processing in synthetic biology.

Virgnija Paliulytė
"Postpartum Ultrasound"

VIRGINIJA PALIULYTĖ, Gražina Stanislava Drąsutienė, Diana Ramašauskaitė, Juozas Kurmanavičius

Introduction: Postpartum ultrasound is an area which has not yet been investigated in Lithuania. Practitioners are continuously concerned with the differences between the normal puerperium and the uterine involution after complicated deliveries: operative evacuation of RPT, caesarean section or postpartum endometritis.

Objectives: The aim of the study is to draw attention to the early postpartum ultrasound and uterine involution period after normal and complicated delivery.

Materials and methods: Longitudinal prospective study. Repeated uterine parameters were measured and the endometrial contents, the diastolic notch, the uterine scar were observed on the 1st, 3rd, 10th, 30th, 42nd, 60th postpartum days. The analysis was performed using SPSS version 21. Multiple ultrasound pictures were collected.

Results: 46 women after normal and 16 women after complicated labor were included to the study. There was no difference between the median uterus parameters and the uterine artery indices between the physiological and pathological groups during the involution period. A more retroverted uterus was found in the pathological group within two hours after labour (72.50 ± 15.77°; p=0.039), while the further angulation trend remained unchanged. No notching of the uterine artery in the pathological group was

observed within two hours after normal labour or on the 10th day. The diastolic notch, irrespective of the group, did not appear in all the postpartum women even after two months following labour. There was evidence of more frequent gas detected in the pathological (44%) rather than in the physiological group (11%).

Conclusions: The same decreasing trend is observed in the main uterine size parameters and the uterine artery indices after normal or complicated labour. The evaluation of the uterine scar healing needs to be equipped with more sonographic parameters than its length, width or view.

Giedrė Šilkūnienė

"Hyperthermia Induced Changes in Viability and Proliferation of Tumorigenic and Nottumorigenic Cells"

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Hyperthermia or hyperthermia in combination with chemotherapy and radiotherapy is widely used for cancer therapy. However, hyperthermia is effective only for elimination of certain types of cancer cells and more detailed knowledge on response of different cells to hyperthermic treatment is needed. Common tests for cell viability are based on estimation of certain cellular function (metabolism based on one substance, plasma membrane permeability, etc.). It is often not clear, whether these functions recover with the time passed after treatment and what is their impact on survival of treated cells. The aim of our work was to compare the effects of hyperthermia (in the range of temperatures used for oncotherapy) on viability of tumorigenic and not tumorigenic cells detected by different methods and to estimate

correlation of results obtained by various viability tests with actual cell survival.

Hyperthermia (HT) (42°C) was applied on tumorigenic (MH22a, PANC1, BXPC3, B16-F1, WEHI- 164, L929) and not tumorigenic (CHO, BHK-21, McCoy) cell lines for 30 min. Immediately after the HT treatment, viability of cells was estimated by calcein-AM or propidium iodide methods. For cell survival colony formation assay was used.

Response to hyperthermia estimated by viability tests was diverse among the analyzed cell lines. Cell staining with calcein-AM showed that 42°C hyperthermia inactivated esterases by 40% in 2 (out of 3) not tumorigenic and 3 (out of 6) tumorigenic cell lines. Meanwhile, plasma membrane permeability evaluation by cell staining with propidium iodide indicated negligible viability changes (up to 15%) nearly in all cell lines. Furthermore, the assay of colony formation revealed that MH22a cells had only 30% survival ratio, although these cells appeared as resistant to HT when evaluated by calceinAM and propidium iodide methods. In contrast, cells B16 which seemed to be very sensitive to HT treatment when stained with calcein-AM and propidium iodide, had 80% survival ratio by colony formation.

Thus, the obtained results indicate that immediate viability tests do not represent cell ability to survive on longer time scales and are less informative in comparison to the colony formation assay.

Andrius Jasinevičius

"Characterization of Human Colorectal Carcinoma HCT116 and SW620 Sublines Resistant to 5-Fluorouracil and Oxaliplatin"

ANDRIUS JASINEVIČIUS, Eglė Kukcinavičiūtė, Daiva Dabkevičienė

Colorectal cancer is the fourth most common oncological disease worldwide. Most colorectal carcinoma patients are treated by surgical removal of the tumor, followed by combinedchemotherapeutic treatment FOLFOX (5-fluorouracil, oxaliplatin and leucovorin), which is the regimen of choice for advanced-stage tumors. Unfortunately, the effectiveness of the chemotherapeutic treatment is limited by intrinsic or acquired chemoresistance of cancer cells.

The aim of this study was to characterize HCT116/FU+OXA and SW620/FU+OXA cell sublines which are derived from HCT116 or SW620 cell lines by continuous cultivation with 5-fluorouracil and oxaliplatin. For that, the comparison of chemoresistant vs. sensitive cells was made in terms of cell morphology, rate of cell adhesion to culture dish, cell cycle duration and degree of chemoresistance.

Vilma Jersovienė

"Human Papillomavirus Infection And Infertility"

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Introduction: The human papillomaviruses (HPV) are sexually transmitted viruses and etiological agents of several human cancers common among men and women worldwide. Recent evidence suggests that HPV infection may affect fertility. In men, HPV infection can affect sperm parameters, specifically concentration and motility.

Aim: To determine the prevalence of HPV among couples attending for the IVF treatment in Santaros Fertility Centre.

Materials and methods: A total of 38 samples (19 men and 19 women) were collected from couples followed for IVF/ICSI treatment in VUH SK Santaros Fertlity Center. HPV-DNA detection was performed by nucleic acid amplification test for qualitative detection and differentiation of high oncogenic risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 DNA in the clinical material (cervical swabs, sperm samples) using real-time hybridizationfluorescence detection of amplified products (AmpliSens HPV HCR genotype-FRT PCR kit, InterLabService).

All semen samples of subjects were collected by masturbation after 3–4 days of abstinence. Semen was assessed according to the World Health Organization (WHO) 2010 guidelines by well-trained

embryologists. Cervical swabs samples from women were taken before ovarian puncture procedure.

Results: A total of 38 samples were collected from couples undergoing IVF treatment. HPV DNA was detected in 29% (11/38) of samples. The prevalence of HPV DNA infection in semen was 32% (6/19), in cervical swabs was 26% (5/19). Sperm quality differs significantly between men with seminal HPV infection and uninfected men: samples positive for HPV were associated with abnormal sperm count and motility (oligoasthenospermia).

Conclusion: This pilot study reveals a significant association between HPV-positive semen and oligoasthenospermia. HPV seminal infections could play an important role in male and in women infertility. These results deserve further attention and additional studies should be conducted with larger cohorts.

Viktorija Zaranko

"The Effect of Hydration on Nanomechanical Response of Porcine Skin"

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Introduction: Skin is a barrier that protects organism from external factors/pathogens and prevents water loss. Various skin properties depend on the humidity of external environment. Knowledge of how humidity affects nanomechanical properties of the skin could be a great interest for pharmacy, medicine and cosmetology.

In this work, atomic force microscopy (AFM) was used to investigate the changes in topography and elasticity of the dermatomed porcine ear skin due to different water activity (a_w) in ambient environment. Nanomechanical and viscoelastic properties of the superficial skin layer, stratum corneum, may vary with the water content. And the response to water loss may indicate the state of skin – healthy intact or damaged (either unhealthy or treated, scarred) skin tissue.

Aim: The aim of this project is to develop a protocol for the use of AFM in a personalized health care system.

Materials and methods: The frozen samples of dermatomed porcine ear (~500 µm in the thickness) were thawed and incubated for 24 hours in three solutions with different water activity, a_w: phosphate buffer (PBS) pH 7,4, a_w=0,992; phosphate buffer/ propylene glycol (PBS/PG) 8/2, a_w=0,936; phosphate buffer/ polyethylene glycol 1500 (PBS/PEG1500) 55/45, a_w=0,946. After incubation, surface topography maps and force-distance curves of samples were obtained either in the plain PBS or in the solution of interest. Mean elastic (Young's) moduli of the samples was calculated from force curves according Hertz model (spherical indenter).

Results and conclusion: Calculated elastic moduli for skin samples, incubated in PBS, PBS/PG and PBS/PEG1500 were 1,23±0,86 MPa, 1.75±0,82 MPa and 1,81±0,70 MPa, respectively. Results show that even small changes in water activity significantly affect stiffness of the skin – with decreasing water activity elastic modulus increases, i.e., skin becomes stiffer.

Rūta Prakapaitė

"Development of an *ex vivo* Porcine Kidney Model for Studying Growth and Virulence of Uropathogenic *E. coli*"

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Introduction: Uropathogenic *E. coli* (UPEC) is considered exceptionally responsible for the significant rates of urinary tract infections and neonatal meningitis. Generally, virulence factors define capability of UPEC to attach to mucosal and urothelial tissue of a host, escape evasion of host defense mechanisms, colonize kidneys and cause disseminated systemic infections. A live host is needed to recapitulate the complex structure of tissues for studying the virulence of pathogens, whereas *in vitro* models are constrained. However, experiments on live hosts are limited because of ethical reasons.

The **aim** was to develop an *ex vivo* porcine kidney model for studying growth and virulence of UPEC and to investigate the interaction between bacteria and renal tissue.

Materials and methods: Explants (±0,5 cm^3) were harvested from fresh porcine kidneys and treated with PBS, supplemented with streptomycin/penicillin G. Afterwards PBS washed renal explants were incubated in RPMI-1640 medium, supplemented with 10%

FBS and ampicillin. The viability of explants was assessed by using Alamar Blue assay. Explants were challenged to $8,5 \times 10^5$ CFU/mL of different UPEC strains (n=9) with diverse serotypes and profiles of virulence genes (*fimH, cnv, iss, ibeA, neuC*). To quantify the number of adhered bacteria, explants were pretreated with Cytochalasin D prior to infection. To quantify the amount of tissue associated and internalized bacteria, Cytochalasin D was omitted.

Results: Renal explants retained their viability after 16 hours of incubation. After incubation, $1,34 \times 10^{10}$ CFU/g of clinical isolate E1 (O:15, *fimH, cnv, iss*) were adhered, but not internalized to the explant, whereas E6 (O:15, *fimH, ibeA*) $1,29 \times 10^{10}$CFU/g associated and $9,45 \times 10^9$CFU/g internalized via other than actin F dependent pathway to the explant.

Conclusion: The successfully developed renal model was shown to be an effective approach to study virulence of UPEC under *ex vivo* conditions.

Mantas Šilkūnas

"Electrochemotherapy Effectiveness in Hepatoma Model *in vitro*: Hypoxia vs Normoxia"

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Introduction: Liver cancer is one of the most common tumors, traditional invasive treatment still faces ineffectiveness or inapplicable. Relatively new approach – combination of cytotoxic

compound and external electrical fields - electrochemotherapy (ECT) could get better disease control. ECT application *in vivo*, revealed lower effectiveness then expected, we hypothesized - extracellular oxygen concentration could be partly responsible. Especially, when physiological aspects (tumor anatomy/location, blood supply et etc.) and ECT specific events (vasoconstriction, oxygen activated bleomycin et ect.) are present.

Aim of the study – evaluate effectiveness of ECT in different oxygen environments.

Materials and methods: Traditionally cultivated mouse hepatoma MH-22A cells was used as model. Detached cell and cytotoxic, membrane impenetrable agent - bleomycin was used for suspension preparation. Prepared cells were permeabilized by applying single, 2kV/cm external electrical field pulse, for 100µs (electroporated), diluted and seeded in petri dish for colony-forming assay. Prepared petri dishes were placed in incubators in 21% oxygen level (atmospheric/normoxia) or 0,2% oxygen level (hypoxia) immediately after manipulation. Effect of hypoxia in compare to normoxia was evaluated in cases of classical chemotherapy (without electroporation) and ECT using different bleomycin concentration.

Results showed, that in case of chemotherapy low bleomycin concentration couldn't penetrate plasma membrane leading to cancer cell high survivability in normoxia. Cell death was reached only when tremendous bleomycin concentrations (range of mIU) were used. In case of hypoxia, MH-22A cell exhibited even higher resistance to bleomycin, LD_{50} could not be reached in investigated range. We believe that, design of experiment, and fast ECT effect, limited to distinguish differences between normoxia and hypoxia in ECT, leading to overlapping of cell viability curves.

Conclusion: Hypoxia plays role in bleomycin chemotherapy in as compared with hepatoma model *in vitro* at atmospheric oxygen concentration, but this could not be seen in the ECT experiments. It is possible, that experiment manipulation time windows and media acclimatization in hypoxic conditions was sufficient to reach maximal ECT effectiveness.

Juta Rainytė

"The Development of Batai Virus-Specific Serological Detection System"

JUTA RAINYTĖ, Justas Lazutka, Rasa Petraitytė-Burneikienė

Introduction: Batai virus (BATV) belongs to *Peribunyaviridae* family of viruses and it is the most abundant pathogenic arbovirus in its *Orthobunyavirus* genus. It is an etiological agent of human and animal diseases. It can cause a febrile disease in humans and it has been associated with a high incidence of abortions, premature births, and congenital defects in ruminants. It is suggested that the middle segment of BATV genome increases the pathogenicity of new viruses that are produced during a reassortment event. Early detection and diagnosis might help lower the spread of BATV and minimize disease-related economic losses.

Aim: The aim of this work was to develop an indirect enzyme-linked immunosorbent assay (ELISA) based on recombinant BATV nucleocapsid (N) protein. The developed BATV detection system was used to screen cow blood serum samples collected in various Lithuanian farms.

Materials and methods: Several eukaryotic and prokaryotic protein synthesis systems were exploited for the generation of the

most suitable BATV antigen. In the end, *S. cerevisiae*-expressed BATV N protein purified under native conditions was used in creating an indirect ELISA for detecting antibodies against BATV in cow blood serum samples.

Results: The prevalence of potentially BATV infected cows among Lithuanian regions is between 20 to 32,5 %. However, some serum samples showed reactivity with a control antigen – recombinant N protein of Schmallenberg virus. Even after removing the dubious samples lest the antibodies against BATV cross-react with the SBV N protein, the BATV prevalence was observed between 5 to 25 %.

Conclusion: This work shows for the first time that there are possibly cows infected with BATV in Lithuania. To validate these results additional experiments must be done: analysis of cross-reactivity of antibodies of BATV-positive samples with antigens of closely related viruses and sequencing the extracted virus RNA from BATV infected cows.

Eigilė Eidėnaitė

"Investigation of 5-Fluorouracil and Oxaliplatin Induced Necroptosis in Colorectal Cancer"

EIGILĖ EIDĖNAITĖ, Eglė Kukcinavičiūtė, Daiva Dabkevičienė, Aušra Sasnauskienė, Violeta Jonušienė

Chemotherapy drugs, 5-Fluorouracil (5-FU) and Oxaliplatin (OxaPt), are widely used for colorectal cancer treatment. However, treatment often fails because of acquired or intrinsic drug resistance. Studies pointed out, that cancer cells which are able to escape from apoptosis become more resistant to cancer

drugs. It is believed that this resistance to apoptosis could be overcome by triggering necroptosis pathway. Necroptosis is the newly discovered caspase-independent programmed cell death pathway, also known as programmed necrosis, which is executed by receptor-interacting protein kinase 1 (RIPK1), RIPK3, and mixed lineage kinase domain-like protein (MLKL).

In this study, we investigated the importance of necroptosis pathway after treatment with 5-FU and OxaPt in human colorectal cancer HCT116 and SW620 cell lines and their 5-fluorouracil resistant and oxaliplatin-resistant sublines. The data on cell viability assays after pre-treatment with necroptosis inhibitor necrostatin-1 (Nec-1) for 2 h, followed by stimulation with 5-FU or OxaPt for 48h and mRNR levels of key mediators and regulators in necroptosis pathway quantified via RT-PCR will be presented.

Aistė Skeberdytė

"DCA in Combination with Salinomycin Exerts Synergistic Anti-Cancer Effect on Colorectal Cancer Cell Lines"

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In the present study we examined a hypothesis that salinomycin, an antibiotic ionophore, might efficiently potentiate the cytotoxic effect of dichloroacetate (DCA), a metabolic inhibitor, on cancer

cells. We used two human colorectal cancer derived cell lines - DLD-1 and HCT116. First, we performed series of dose response experiments in 2D cell culture in mono- and combination therapy and evaluated effects, based on Chou-Talalay method. This analysis revealed that salinomycin in combination with DCA acted synergistically in both cell lines. Secondly, in order to recapitulate the in vivo tumour architecture, we tested doses, selected from 2D experiments, in 3D multicellular spheroid culture in mono- and in combination therapy. In such conditions, the effect of salinomycin and DCA was additive in DLD-1 and synergistic in HCT116 cell lines. Further, we demonstrate that synergistic effect of compounds might be related with inhibitory effect of DCA on multidrug resistance proteins (MRPs) and with reduction of intracellular pH that in cancer cells is elevated. The activity of MRPs as well as potency of certain drugs is pH-dependent. Finally in order to disclose the rationale for sensitivity variations to combination therapy between two cell lines, we have investigated gene expression profiling of DLD-1 and HCT116 cell lines in 2D and 3D cell cultures and presume that decreased sensitivity of DLD-1 cells to combination therapy could be attributed to increased stemness of DLD-1 cells in 3D cultures compared to HCT116 cells.

Kristina Stuopelytė

"Blood-circulating Androgen Receptor Variants as Markers for Progression and Response to Treatment in Prostate Cancer Patients"

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Despite prostate specific antigen-screening and modern treatment techniques, prostate cancer (PCa) is the second most prevalent oncologic disease and the fifth leading cause of cancer-related death among men worldwide. The particular treatment for castration-resistant PC (CRPC), the most aggressive form of the disease, is expensive and suitable for only a certain part of patients. A significant part of patients already are resistant or develop it in the course of treatment. Blood-circulating tumor cells carry PCa-specific transcripts, like androgen receptor variants (AR-Vs), which reflect the situation in tumor, and could also serve as the non-invasive PCa monitoring tools. The aim of our study was to develop novel AR-Vs-based molecular tool to non-invasively diagnose, predict PCa progression and response to treatment directly from the blood sample.

127 PAXgene RNA blood samples were collected from 92 CRPC patients (33 cases have serial samples) in National Cancer Institute and VU Hospital Santaros Klinikos during 2016-2017. The response to treatment was rated as positive (*Pos*, N=80), biochemical

recurrence (*BCR*, N=14) or death (*Death*, N=12). For the detection in patients' blood, custom made TaqMan assays for AR-V1, V3, and V7 were used for target-specific reverse transcription, pre-amplification and real-time PCR.

AR-V1, -V3 and -V7 were detected in 17 (14%), 59 (46%), and 86 (68%) samples, respectively. 25 samples were AR-Vs-free, and 12 contained all variants tested. After the cases were stratified according to response to treatment, AR-V3 was detected significantly more frequently in *BCR* vs *Pos* (FC=5.4, P=0.0336), whereas V1 – in both, *Death* vs *Pos* (FC=60.0, p=0.0010) and *Death* vs *BCR* (FC=69.4, P=0.0400) comparisons. Furthermore, V1 and V3 were independent prognostic markers for disease progression separately (P=0.0032 and P=0.0072, respectively) and in combination (P=0.0275).

Blood-circulating androgen receptor variants can serve as the non-invasive biomarkers for sensitive and specific prediction of BCR and response to treatment.

Gertrūda Pėtelytė

"Cross-Species Transmission of *Puumala* Virus from Rodents to Domestic Swine"

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Introduction: *Puumala* virus (PUUV) is widely distributed European hantavirus that can cause hemorrhagic fewer with renal syndrome named nephropathia epidemica. This virus is usually transmitted to humans through the contact with infected bank voles (*Myodes glareolus*). Moreover, epidemic occurrence of PUUV clinical cases has been increasing in Europe revealing the highest prevalence in Germany. Surprisingly, occasional cases of hantavirus infection are reported in non-rodent species indicating possible virus transmission to mammals most likely through the contact with hantavirus-infected rodents. The insufficient knowledge of hantavirus reservoir species encouraged us to determine the spillover of rodent-borne PUUV in domestic swine.

Aim: The aim of this study is to assess cross-species transmission of rodent-borne *Puumala* virus to domestic swine.

Materials and methods: *Saccharomyces cerevisiae* was employed to synthesize histidine-tagged recombinant nucleocapsid (N) proteins of PUUV and *Tomato spotted wilt* virus (TSWV). Proteins were purified under denaturing conditions and used in serological assays. Blood serum samples of domestic swine were tested for

antibodies against PUUV N protein by indirect ELISA, Line blot and Dot blot methods.

Results: A total of 377domestic swine sera from Baden-Württemberg federal state of Germany collected between the years of 2008-2010 have been tested by indirect ELISA. The most reactive sera were additionally tested by Line blot. 13 sera confirmed previously reported reactivity with PUUV N antigen. For localization of PUUV-specific epitopes in selected 13 serum samples, a series of truncated histidine-tagged PUUV N proteins were used. Results indicated different immunogenic regions localized in PUUV N antigen.

Conclusion: Out of 377 swine serum samples, antibodies from 13 specimens recognized PUUV N antigen in ELISA and Line-blot. These findings indicate probable cross-species transmission of rodent-borne PUUV to domestic swine and raise more questions about hantaviral host switch events. However, for more accurate results more tests need to be done.

Agnė Šeštokaitė

"Quantitative *DAPK1* and *p16* Promoter Methylation Analysis in Non-Small Cell Lung Cancer Patients"

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Despite advances in treatment means, lung cancer (LC) still remains the most common cancer worldwide. LC is known for the late presentation mainly attributed to the lack of biomarkers for screening and routine monitoring. Long term smoking is one of the biggest risk factors. Smoking can cause DNA methylation changes in promoters of tumor suppresson genes in early stage of LC, which can be used as biomarkers for LC prevention.

The study was focused on evaluating promoter regions of *DAPK1* (8 CpGs) and *p16* (7 CpGs) methylation frequency (MF) and intensity (MI) in primary non-small cell lung cancer (NSLC) samples.

Analysis was conducted in 100 tumor (T) (and 101 nontumor, NT) tissue specimens, however 68 T (92 NT) samples for DAPK1, and 100 T (101 TN) for *p16* met the quality requirements. Extracted DNA was bisulfite-treated, multiplied by PCR, checked by gel electrophoresis and pyrosequenced.

Comparison of *DAPK1* methylation in T vs NT revealed that MFs and MIs were significantly higher in tumors for *DAPK1* (54.4% vs 29.3%, p=0.0019 and 5.3% vs 2.2%, p=0.0175 respectively) and for *p16* (58.0% vs 32.7%, p=0.0004 and 5.4 % vs 1.4%, p<0.0001, respectively). After comparing MFs and MIs between patients' groups divided according to their age, gender, type of tumor, smoking status, years of smoking and number of cigarettes per day, MI of *p16* PR was 2-fold higher in NSLCs from patients who smoked >20 cigarettes per day (p=0.0456). Moreover, MIs comparison between separate CpG positions revealed that 5th MI was higher (8.9%, p<0.0001) and 8th lower (2.8%, p<0.0001) methylated than the rest CpGs for *DAPK1*, and 7th CpG MI was significantly higher (7.2%, p<0.0001) for p16.

In conclusion, more frequent and more intense methylation of *DAPK1* and *p16* in NSLCs suggest possible role of these epigenetic changes in lung carcinogenesis.

Indrė Pauraitė

"The Effect of Fractionated Ionizing Radiation on Human Breast MCF-7 Cancer Line Cells"

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Breast cancer is one of the most common malignancies amongst women all over the world. Nowadays gamma radiation therapy is the main method of treatment used against breast cancer, nevertheless radiotherapy affects both normal cells as well as cancer cells. Furthermore radiation therapy is becoming less effective method of treatment because there is a considerable amount of evidence, that cells which are identified and characterized as cancer stem cells (CSCs) with stem-cell-like properties develop an adaptive response to radiation. CSCs are non-specialized cells that are capable of uncontrollable proliferation, self-renewal and differentiation in order to sustain tumor growth and progression. Moreover CSCs have great potential to be used as a target for cancer therapy mainly of their ability to resist radiation therapy.

The main purpose of our research was to determine fractionated ionizing radiation effect on MCF-7 cells morphology, viability, growth rate and accumulation of CdSe/ZnS carboxyl-coated quantum dots.

In our study human breast adenocarcinoma MCF-7 cells were treated with fractionated ionizing radiation dose of 3x4Gy using Varian Clinac 600C/D linear accelerator. Viability and growth of cells were determined using Adam-MC automated cell counter. MCF-7 cells morphology and accumulation of CdSe/ZnS quantum dots were evaluated using Nikon Eclipse TE2000-U microscope with the confocal laser scanning system C1si.

Study results revealed that ionizing radiation triggers morphological changes in MCF-7 cells. There was a significant changes in cells sizes and in sizes of cell nuclei. Furthermore we determined that after the treatment with ionizing radiation, growth rate and viability of irradiated cells decreased, while control cells were stable during all experiments. Finally, we observed that accumulation of quantum dots in irradiated cells were heterogeneous, whereas in control cells accumulations was homogeneous.

Overall it is essential to understand the importance of the features that contribute to breast cancer stem cells radiosensitivity in order to develop new methods of treatment, which could help to eliminate cancerous formations, but at the same time would have the least toxic effect on normal cells.

Ilona Trockaja

"Identification of New Glioblastoma Biomarkers in Modern Cell Culture Models and Biological Samples"

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2 Life Science Center, Vilnius, Lithuania

Glioblastoma is one of the most aggressive malignant brain tumors and despite the standard surgery, radiation and chemotherapy, affected individuals has a median survival of 12-15 months following diagnosis. In order to understand the genetic mechanisms of tumor initiation and progression lots of different model systems are employed, but there are still lots of open questions left.

The aim of this study was to evaluate the changes in the expression of gene and miRNA in glioblastoma U-87 cell line 2D and 3D cultivated cell cultures and glioblastoma tissue in order to find out the potential biomarkers, which have an important role in glioma initiation, progression and invasion.

The genome-wide analysis using a microarray-based technique and NGS revealed the gene expression and miRNA profiles of 2D and 3D cultivated glioblastoma cell line U-87 and identified genes and miRNAs, which can be important in glioblastoma initiation and progression. The KEGG analysis pointed the main genes and miRNA targets function groups which were validated in model system and human glioblastoma specimens using RT-PCR proving its importance as a new potential molecular biomarker.

Tadas Saulėnas, Milda Adomaitytė

"Factors Related to the Strength of Motivation Among Medical Students"

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Introduction: Studying medicine requires greater effort; a long-term motivation is important for academic achievements. It is known that motivation correlates with organizational policy, coworkers' relations, personal life. There is no research on the strength of motivation among Lithuanian medical students.

Aim: To investigate the relations of factors to the strength of motivation to study among Lithuanian medical students.

Materials and methods: Overall 818 medical students (51.6 % from Lithuanian University of Health Sciences and 48.4 % -from Vilnius University) anonymously completed sociodemographic and The Strength of Motivation for Medical School (SMMS) questionnaires. In the SMMS the scores of 3 subscales- willingness to sacrifice; readiness to start; persistence and total (mean) score were used (5 questions in each subscale, scoring 1 to 10). Bioethics Committee approval № BEC-MF-388. Data was analyzed using the SPSS 23.0. $P<0.05$ was considered statistically significant.

Results: The age range of the study participants was 18-35 years (mean 21.17, SD 2.05), 75.2% were women. The plans to work abroad

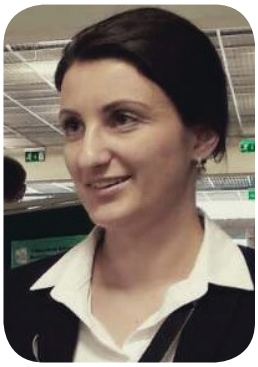
reported 30.3% of students. Mean motivation score of willingness to sacrifice was 6.29 (SD 2.00), readiness to start 6.86 (SD 2.23), persistence 6.94 (SD 1.66), total 6.7 (SD 1.6). Higher strength of motivation reported students, who were motivated by colleagues and lectures (Z=6.14; p<0.001), not pressured by lectures (Z=5.452; p<0.001). Older age related to lower motivation of readiness to start studying (r = -0.072; p=0.038). Higher motivation related to the feeling happiness about studies (r=0.318; p<0,001), learning in university as valuable (r=0.255), duration of self-studies (r=0.073; p =0.004), having priorities in own life (Z=3.839; p<0.001) and general sense of happiness (r=0.224; p<0.001).

Conclusion:

- Medical students were moderately motivated.
- One third of students reported plans to the job abroad.
- Positive relationships with lecturers, individual preferences and sense of happiness related to greater motivation, while an older age -with lower motivation.

Ieva Sadzevičienė

"Analysis Of Promoter DNA Methylation And *TP53* Mutation Status in Breast Tumors"



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Introduction: Breast cancer (BC) can be effectively treated at early stages, with therapy specific for BC subtype. Nowadays, clinical-pathological and immunohistochemical patients' characteristics

are used to predict BC progression or clinical outcome. However, these parameters often lack accuracy. Promoter methylation of tumor suppressor genes (TSGs) and *TP53* mutations have been shown to occur at early stages of carcinogenesis and, therefore, might be suitable for timely diagnostics and determination of BC subtypes.

Our **aim** was to evaluate promoter methylation of selected TSGs and *TP53* mutation status according to molecular BC subtypes.

Methods: In total, 124 BC samples and 29 noncancerous tissues were included in the study. Methylation of 15 TSGs (*p14, p16, RUNX3, DAPK1, GSTP1, RARB, MGMT, ESR1, ADAMTS12, APC, RASSF1, NAALAD2, MT1E, MT1G, and PRKCB*) were analyzed using methylation-specific PCR. *TP53* mutation status were evaluated by means of single-strand conformation analysis and validated with Sanger and/or next-generation sequencing.

Results: *APC* and *RUNX3* were more frequently methylated in HER2-positive BC (64% and 68%, respectively) comparing to luminal A (LA; 55% and 47%,), luminal B (LB; 50% and 58%), and triplenegative BC (TNBC) subtypes (44% and 6%; all $P < 0.05$). Furthermore, methylation of *PRKCB, ESR1, RUNX3*, and *MT1E* was also specific to TNBC cases (44%, 31%, 6%, and 50%, respectively; all $P < 0.05$). *TP53* mutations were frequent in TNBC cases (81%) and were less commonly detected in LA, LB, and HER2-positive BC subtypes (9%, 38%, and 30%, respectively; all $P < 0.05$). Various associations were also observed between TSG methylation or *TP53* mutation status and other clinicalpathological and immunohistochemical parameters.

Conclusion: Our study revealed significant associations of TSG methylation and *TP53* mutations in TNBC, which might be utilized for better characterization of molecular BC subtypes.

Gabija Julija Rudzikaitė, Eglė Olekaitė

"*In Vitro* Activity of Amphotericins and Triazoles in Combination with Polyphenols, Pentacyclic Triterpenoids and Fatty Acid Derivatives Against Biofilms of *Candida Albicans*"

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Introduction: *Candida* species cause 6,3% of all hospital infections, however, they are responsible for 22% of systemic candidemia cases which result in 19-24% mortality rate. *Candida* biofilms are more resistant to drugs in comparison with planktonic forms. For that reason, it is important to seek for new treatment opportunities. Catheters, immunosuppression and prolonged antibiotic use are the main risk factors of biofilms producing *C. albicans* infections.

Aim: To investigate the antimicrobial interactions between polyphenols, triterpenoids and fatty acid derivatives in combination with Amphotericin B (AmB) and Fluconazole (Flu) against *C. albicans* biofilms.

Materials and methods: Biofilm production in *C. albicans* was quantified by crystal violet assay. CLSI (M38-A2) broth microdilution methodology was used to determine MICs for Flu, AmB, rosmarinic acid (R), pentacyclic triterpenoid (betulinic acid, B), fatty acid (oleic

acid, O) against 3 isolates of planktonic and biofilm associated *C. albicans*. Viability of *C. albicans* was determined using *AlamarBlue* assay. Viable *C. albicans* quantified by serial dilution and plating. Synergistic relations of drugs were analyzed by fractional inhibitory concentration (FIC) determination.

Results: Both planktonic and biofilm associated *C. albicans* showed high level resistance to Flucanozole (MIC>200 µg /mL). Planktonic forms and biofilms were both equally sensitive to AmB (MIC 0.1 µg/mL). AmB versus its combination with rosmarinic acid ($MIC_{AmB/R}$= 0.01/5.7 µg/mL) had significantly decreased biofilm formation ($OD_{600\ nm}$ 1.291 versus 0.866, p=0.0119, α=0.05), which refers to synergistic drug relation (ΣFIC=0.10099). Moreover, rosmarinic acid in combination with Fluconazole ($MIC_{Flu/R}$= 20/570 µg/mL, $OD_{600\ nm}$ 0.585, p=0.0024) showed synergistic impact on biofilms (ΣFIC=0.199). Other single and combined antifungals did not show statistically significant impact on *C. albicans* biofilms (p=0.1307-0.1448).

Conclusion: Synergy of Amphotericin B combination with rosmarinic acid may be successfully applied to prevent risk patients from severe infections, caused by biofilms producing *C. albicans*.

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LIFE SCIENCES CENTER

The campus of Vilnius University at Saulėtekio Avenue was recently expanded by a new building of the Life Sciences Centre (LSC) covering the total area of 24 thousand square meters.

LSC operate on the basis of an agreement between the three academic branch units – Institutes of Biochemistry and of Biotechnology and the VU Faculty of Natural Sciences.

Activities of the LSC facilitate scientific research, studies and technological development in the fields of biochemistry, biotechnology, molecular biology, genetics, neurobiology, molecular medicine and other related sciences.

The LSC is a part of the 'Santara Valley' project. Together with the 'Sunrise Valley' project they both seek to stimulate a breakthrough in research development and the commercialization of research. Both projects have been initiated by VU in cooperation with other national institutions.

The 'Sunrise Valley' project concentrates on the research potential in the field of laser and light technologies, materials science, nanotechnologies, semiconductor physics and electronics; whereas the project 'SantaraValley' focuses on biotechnology, bio-pharmacy, molecular medicine, innovative medical technologies, information technology, ecosystems and safe environment.

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The book of Abstract layout by Justinas Kavoliūnas, Mantas Sakalauskas, Inga Kričenaitė.

Host of the conference: Povilas Marma.

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